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INFLUENCE OF TEMPERATURE ON OPTIMUM HYDROGEN-ION CONCENTRATION FOR THE DIASTATIC ACTIVITY OF MALT[1]

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The terms "optimum hydrogen-ion concentration" or "optimum pH" for different enzyme reactions are frequently encountered in the literature without reference to the temperature at which the determinations were made. With reference to diastase, the more recent of these determinations have been made at or near 25°C., that being a convenient temperature for laboratory experiments. No one seems to have questioned the validity of these reported values for temperatures other than that at which they were determined.

As many industrial processes are conducted at temperatures higher than those referred to, it becomes important to know definitely to what extent the pH values reported as optimum can be considered applicable under different temperature conditions.

Rumsey (1) and Collatz (2) have recently published very extensive studies of the diastases of wheat and malt, respectively. These authors reviewed the previous literature and reported values for the optimum pH of 4.7 to 4.8 for wheat diastase and 4.26 for malt diastase. Rumsey worked at 27°C., while Collatz studied the diastatic activity at 25°C. Sherman et al (3) had previously reported 4.4 to 4.5 as the optimum pH for malt diastase at 40°C.; however, an examination of their experimental data would indicate that 4.8 might just as properly be considered optimum. The latter figure closely agrees with data to be presented in this paper.

[1] The authors are indebted to P. D. Henderson for much of the analytical data in this paper.

Recently, in this laboratory, we had occasion to conduct some diastatic operations with mixtures of wheat and malted barley flours at increasing temperatures up to 70°C. It was noted that at the higher temperatures an increase in acidity beyond the normal pH of approximately 6.0 tended to inhibit the diastatic action. This was contrary to expectations based upon the reported optimum (pH 4.5 to 4.8) and indicated the possibility that the optimum hydrogen-ion concentration changed with the rise in temperature, it being natural to suppose that the greater activity of the acid at a higher temperature would have the same effect as an increase in hydrogen-ion concentration.

In order to test this point the optimum hydrogen-ion concentration was determined at different temperatures, using water suspensions of a mixture of wheat and malted barley flours. In each case 200 cc. of water containing different amounts of dilute acid or alkali was measured into 250 cc. Erlenmeyer flasks and brought to the desired temperature in a water thermostat. Twenty grams of the flour were then thoroly dispersed in the liquid and the flasks kept in the thermostat, with occasional stirring, for exactly 60 minutes. Fifty cc. of the suspension was then pipetted directly into 250 cc. volumetric flasks containing 3 cc. of a 15% sodium tungstate solution and about 100 cc. of cold tap water. After acidulating as recommended by Rumsey (1), the contents of the flasks were made up to volume, centrifuged and filtered. Twenty-five cc. aliquots were used for determining the reducing power, thus being roughly equal to 0.5 gram of the original flour mixture. As the values obtained are of purely relative importance, no attempt has been made to correct the figures for volume displacement. It has also been thought best to plot the actual weights of Cu_2O obtained rather than to convert them into doubtful percentages of glucose or maltose; either conversion would be purely arbitrary and therefore add nothing to the point in question. The remainder of the original digestion mixture was used for the electrometric determination of the hydrogen-ion concentration.

The results obtained are given in the following table and are graphically expressed in Figure 1.[2]

[2] While raw flour was used in these experiments the authors recognize that the conversion figures would be much larger had a modified or gelatinized starch been used. However, experiments made in the course of the investigation showed the optimum pH at 25°C. to be the same whether the starch remained raw or had previously been gelatinized and then cooled to 25°C.

TABLE I

HYDROGEN-ION CONCENTRATION OF FLOUR AND WATER MIXTURES AND THE CORRESPONDING REDUCING POWER OF THESE MIXTURES AFTER 60 MINUTES DIGESTION AT DIFFERENT TEMPERATURES

No.	pH	25°±0.5 Mgms. Cu_2O per gm. of flour	pH	45°±0.5 Mgms. Cu_2O per gm. of flour	pH	60°±1.0 Mgms. Cu_2O per gm. of flour	pH	69°C.±1.0 Mgms. Cu_2O per gm. of flour
1	3.97	33.4	3.58	19.8	3.72	19.2	3.67	45.0
2	4.19	38.4	4.11	42.4	4.47	82.6	4.24	59.2
3	4.33	47.4	4.67	71.0	5.06	466.4	5.06	222.6
4	4.48	45.2	4.99	85.0	5.70	569.8	5.71	462.4
5	4.80	43.0	5.33	81.2	5.99	558.2	5.87	473.8
6	5.17	41.2	5.73	65.0	6.76	414.6	6.05	487.4
7	5.58	34.6	6.07	50.4	7.52	147.2	6.47	415.2
8	....	....	6.46	24.0	....	.....	7.13	137.2

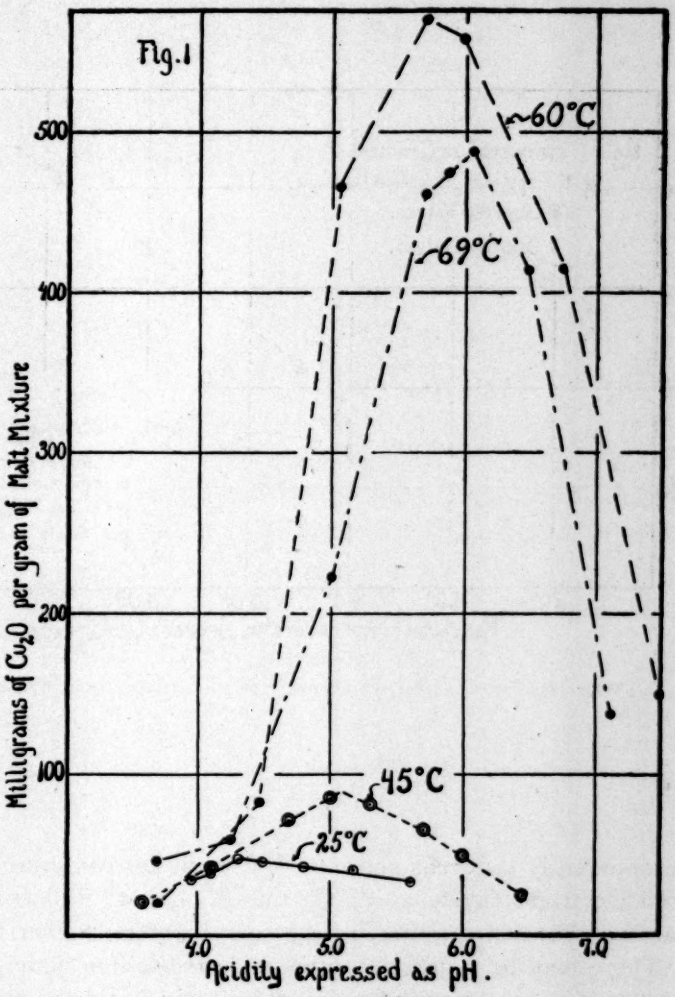

Fig. 1. Influence of Temperature on the Optimum Hydrogen-ion Concentration for Diastatic Activity of Malt

Results of diastatic enzyme experiments performed at 25 to 30°C., are evidently not applicable to work carried on at a different temperature. It is apparent from our data and curves that the optimum hydrogen-ion concentrations for diastatic enzyme activity, and possibly for enzyme activity in general, varies with the temperature. The optimum pH changes from about 4.3 at 25°C., to beyond 6.0 at 69°C.

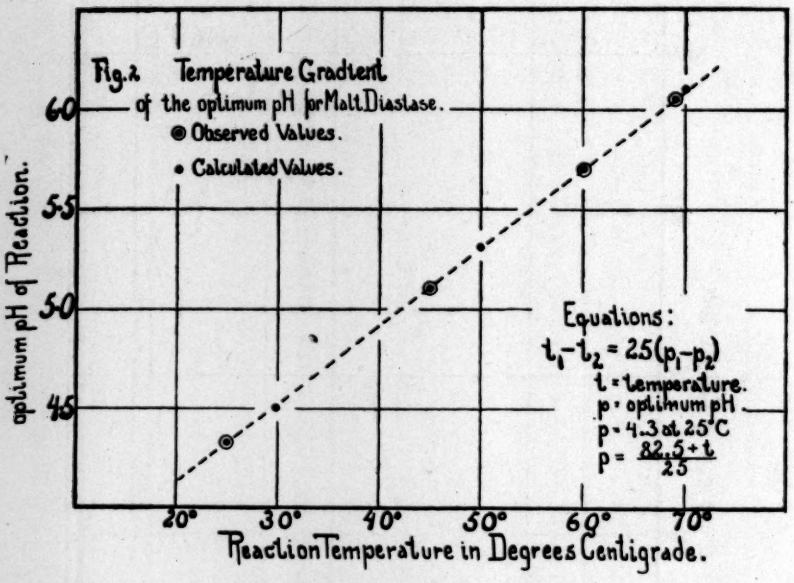

Fig. 2. Temperature Gradient of the Optimum pH for Diastatic Activity of Malt

The apparently different optimum hydrogen-ion *concentrations,* as measured electrometrically at 25°C., may, however, well correspond to equal hydrogen-ion *activities* at the corresponding higher temperatures. There may be a definite optimum *hydrogen-ion* activity, could we measure it at the temperature of the reaction, and the apparently

low acidity needed at high temperatures may at these temperatures represent a hydrogen-ion concentration equal in activity to the greater concentration necessary at 25°C.

It is also important to note the much greater influence of small changes in pH at the higher temperatures. The influence of different acidities at 25°C., so noticeable when plotted on a different scale, becomes almost negligible when compared with the changes experienced at 60°C.

The construction of curves from which the optimum pH for any temperature within the active range of an enzyme can be deducted, should prove of considerable value to enzyme workers in general. While only four temperatures have been covered in this investigation, a study of the temperature gradient revealed by the four points obtained is nevertheless highly instructive. Figure 2 shows the optimum pH values plotted against the corresponding temperatures.[3]

The optimum pH ($\log. \frac{1}{C_{H^+}}$) *appears to be a linear function of the temperature.*

When the different observed values are substituted into the equation for the straight line:

$$(1) \quad t_1 - t_2 = m\ (p_1 - p_2)$$

Where t_1 and t_2 are the temperatures in degrees centigrade, and p_1 and p_2 the corresponding optimum pH values, we arrive at a value of

$$m = 25.$$

Now using the equation

$$(2) \quad (t_1 - t_2) = 25\ (p_1 - p_2)$$

and substituting into it the values of 25°C. and the corresponding optimum 4.3, we get an equation which reduces to

$$(3) \quad p = \frac{82.5 + t}{25}$$

where p is the optinum pH at any temperature, t.

Making use of this equation the optimum pH for any desired temperature may be calculated. A number of values thus calculated have been plotted on Figure 2, and appear to agree exactly with the observed values.

To what extent this equation is of general application is not apparent from present data. It would be of interest to test the equation on diastases from different sources, as well as on other enzyme preparations.

[3] The figure 5.1 for the optimum pH at 45°C. is used because an inspection of Figure 1 shows 5.1 to be nearer correct than 4.99 as given in Table I.

These changes observed may be explained by the theory of "hydrogen-ion catalysis by moderately strong and weak acids" as put forward by Rice (4). The highly buffered solutions necessarily used in enzyme investigations are essentially equivalent to the weak acids considered by that author. If the observed shift of optimum pH accompanying rise of temperature is due merely to this increased concentration of active hydrogen ions, then our equations should hold not only for any diastase but should apply to enzyme studies in general. In this connection it is of interest to note that Nelson and Bloomfield (5) have recently shown a similar "relation between temperature and the critical hydrogen-ion concentration for inactivation of invertase." According to their Figure 4, the shift of the critical pH between 30° and 50°C. is 0.8 of a pH. According to our equation (2) the difference should be 20/25 or 0.8 of a pH, a very striking agreement. These authors failed to find any difference in the optimum pH for invertase activity at 25° and 35°C. The difference to be expected is only 10/25 or 0.4 of a pH, and as this shift comes on a rather flat portion of their curves, the failure to notice such change need not be considered an argument against the theory.

Malt diastase has been considered to be made up of two or three different enzymes. H. C. Gore (6), in connection with his studies on maltose production, reports the optimum pH for liquefaction to be about 6.0, while saccharification is best at 5.0. It is interesting to note that the liquefaction was carried on near 70°C., while the saccharification was performed at 45° to 50°C. These values agree quite well with our curves and show therefore no real difference in optimum pH between the liquefaction and saccharification processes. The temperature employed accounts for the apparent difference.

In our own work the apparent liquefaction, at higher temperature (69°C.), as shown by the tendency of the gelatinized starch and solid particles to settle, showed a striking agreement with the saccharification figures for the same series. Indications were that the two processes, whether activities of the same or of different enzymes, have the same optimum pH. More work is needed on this point.

Summary

1. Attention is called to the need for recognizing the importance of temperature in reporting optimum pH values for enzyme activities.

2. It is shown that the optimum hydrogen-ion concentration for malt diastase, as measured at 25°C., varies from pH 4.3 to 6.0 as the temperature is raised from 25° to 69°C.

3. It is suggested that this change is due to the increased activity of the hydrogen ions present and that these apparently different pH measurements would represent approximately equal hydrogen-ion *activities* if measured at the temperature of the reaction.

4. Data are presented indicating that the optimum pH is a linear function of the temperature agreeing with the straight line equation

$$t_1 - t_2 = m\,(p_1 - p_2)$$

Where t_1 and t_2 are reaction temperatures and p_1 and p_2 the corresponding optimum pH values. This equation is shown to reduce to

$$p = \frac{82.5 + t}{25}$$

from which the optimum pH for any given temperature can be calculated.

5. It is further pointed out that at the higher temperatures the apparent liquefaction curve follows the saccharification curve closely, both processes apparently showing the same optimum pH.

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SOME CRITICAL CONSIDERATIONS OF THE GLUTEN WASHING PROBLEM[1]

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Introduction

When a normal wheat flour is doughed and the dough is allowed to set for some time it is possible to wash out most of the starch and soluble constituents under a stream of water, leaving the coherent gluten behind.

Gluten washing is practiced for two purposes. The weight of any gluten is a fair index to the protein content of the flour. What is more significant, the cereal chemist is able to obtain an indication of flour strength from the physical properties of the washed-out gluten. Many investigators have urged very properly that since nitrogen determinations by the Kjeldahl method are more exact and yield concordant results in the hands of any well-trained analyst, protein content should be calculated from nitrogen content rather than from dry gluten content. However, no simple objective method for evaluating gluten quality has yet come into use which is equal in value to the "feel" of gluten in the hands of an experienced operator. These two factors, quantity and quality of gluten, lose none of their significance because they fail to tell the whole story of flour strength. There are many other factors. Among them, as has been pointed out by Collatz (1922), the diastatic value is a third highly important factor influencing flour strength.

As gluten washing remains important not only because of the index to gluten quality which it furnishes, but to a lesser extent because some idea of protein content is gained, it seems well to inquire whether the method of washing gluten is subject to further refinement. Many investigators have studied the gluten washing problem. The conclusion which is frequently reached is that gluten washing is so inaccurate a procedure that it should be dropped. Yet the fact remains that gluten washing is still commonly employed by cereal experts in evaluating flours.

Gluten washing has been a common procedure for at least 50 years. Lüers (1919) has stated that in 1750 Beccari observed that gluten

[1] To be submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, to the Department of Chemistry, Stanford University.

could be washed from wheat flour. That a gummy substance could be obtained by chewing wheat berries was no doubt known long before.

Consideration of the gluten washing process suggests that there are at least ten important factors which should be considered. These are as follows:

1. Length of period dough is allowed to set.
2. Length of period gluten is allowed to set.
3. Temperature.
4. Length of washing period.
5. Mechanical manipulation.
6. Nature of wash water.
7. Hydrogen-ion concentration of flour.
8. Concentration and kind of electrolytes in flour.
9. Gluten quality.
10. Gluten quantity.

It may be well to review the significant literature bearing on the rôle of each of these factors in gluten washing.

It is well known that after flour is doughed a certain length of time is necessary for the gluten to develop. Balland (1883) studied the development of gluten in dough. He found that gluten did not develop and could not be washed from flours which had been heated to too high a temperature. The enzymic theory of blood coagulation had been advanced some time before and it was proposed by Weyl and Bischoff (1880) that enzymes were involved in gluten formation. While the evidence against enzymic action is not wholly conclusive, it seems highly probable that the formation of gluten is simply a process of imbibition or swelling. The harmful effect of high temperature may be due simply to coagulation of the glutenin. That it takes time for maximum imbibition to be reached is not astonishing. The time required to develop the gluten is probably merely the time necessary to allow the water and the flour components, particularly glutenin and gliadin, to establish proper equilibrium. Lüers (1919) considers that gliadin is the protective protein of gluten and that it forms within the protein phase the dispersing agent for the less reactive glutenin.

Arpin (1902) found that if a dough is allowed to lie for four hours the yield of moist gluten is considerably increased while the yield of dry gluten is unchanged. Kepner (1914) found that the length of time the dough is allowed to stand increases the percentage of wet gluten up to eight hours. However, he points out that high patents, old flours, and low grades are exceptions. In these the yield of dry gluten is unaltered except in low grades, where there is some loss. The procedure of the Association of Official Agricultural Chemists

(1920) for gluten washing prescribes that the dough be allowed to set one hour. There seems to be no valid basis for altering this empirical procedure.

While Kepner (1914) reported that washed glutens do not change weight even if allowed to stand for 24 hours in fresh wash water, there appears to be no experimental basis for the procedure of the Association of Official Agricultural Chemists of allowing the washed gluten to stand in the wash water for one hour before weighing. At least it seems unlikely that it is necessary to adhere as closely to this procedure as to the one-hour period the dough is required to stand preliminary to washing.

It is a common observation that the yield of wet gluten increases with the temperature of the wash water. There is disagreement as to the yield of dry gluten under these conditions. Arpin (1902) used water at 5°, 15°, and 25° and obtained 23.98, 25.26, and 26.42 per cents of wet gluten and 7.83, 8.08, and 9.24 per cents of dry gluten respectively. Kepner (1914) failed to find distinct differences in the yield of dry gluten obtained at 15° and 25°. It would seem advisable therefore to specify the temperature at which gluten washing is to be done. No doubt one factor responsible for variable gluten results is that tap water may vary in temperature from 5° to 25°.

As pointed out by Kepner (1914) the length of the washing period has a decided effect on the yield of both wet and dry gluten. According to Norton's analyses (1905) gluten is a mixture of gliadin, glutenin, globulin, starch, lipoid, fiber, and mineral substances.

He reported the following as the average composition of a crude gluten from a sample of mixed straight durum wheat flours:

	Per cent in crude gluten	Per cent recovered in crude gluten from flour
Total protein (N × 5.7)	80.91	13.39
Ether extract	4.20	0.69
Fiber	2.02	0.34
Ash	2.48	0.41
Carbohydrates other than fiber	9.44	1.56
Total	99.05	16.39
Crude gluten in flour	.....	16.55
Total protein in flour (N × 5.7)	.....	15.25
Constituent proteins of gluten:		
Gliadin	.....	39.09
Glutenin	.....	35.07
Globulin (soluble in 10% salt solution)	.....	6.75

Olsen (1912) found a much lower value for the total protein content of crude gluten. Glutens from 24 flours had an average total protein content (N × 5.7) of 72.79%.

It is interesting to note that gluten retains such a relatively large proportion of ether extract, fiber, and ash. Obviously, varying proportions of the non-protein constituents will be removed as the length of the washing period is varied. Norton's analyses show that all of the carbohydrate is ordinarily not removed in gluten washing. Gerum and Metzer (1923) have concluded that after prolonged washing gluten becomes starch free. Such a gluten when dried had the following composition: H_2O, 0.45; N × 5.7, 89.4[3]; ether extract 0.26; crude fiber, 0.58; P_2O_5, 0.346. On account of the difficulty in removing the starch from gluten, they suggest that in doughing flour the starch swells to a certain extent and in this colloidal state, together with the gluten-forming protein, becomes a part of the adsorption complex. No doubt one of the principal reasons for variable results is simply that larger percentages of carbohydrates are removed as the washing period is prolonged. This phase of the question seems to merit further investigation.

The mechanical manipulation of washing the gluten as well as of doughing the flour must be carried out according to a carefully prescribed method. Kepner (1914) has emphasized the importance of obtaining an absolutely uniform dough. When an excess of water is used in doughing, he found, the yield of wet gluten is increased altho the dry gluten is unaffected.

Probably the factor in gluten-washing which has been controlled least by different investigators is the nature of the wash water. Tap water almost invariably is used. Yet the temperature of tap water may range from 5° to 25°. Its content of solids may vary from practically none to 500 parts per million. Its hydrogen-ion concentration may vary widely. Duval and Dumaraud (1923) have recently reported that the hydrogen-ion concentration of a river water changed from a pH of 7.1 to a pH of 8.0 from the source of the river to its mouth.

The nature and concentration of both the anions and cations may vary within wide limits. "Permanent" hardness is due to such substances as calcium sulphate. "Temporary" hardness is chiefly due to calcium bicarbonate. The former type of water has little buffer value; the latter has decided buffer value.

Professor W. H. Sloan, of the Department of Chemistry, Stanford University, kindly has furnished the following analyses of water from the two wells from which the local tap water is obtained. These

[3] The figure 89.4% for protein content is ours and is calculated from the value they give in which the factor 6.25 was employed. The factor 5.7, however, is now generally accepted as more accurate. It thus appears that this gluten contains nearly 10% of some unidentified material.

analyses were made early in 1923 and since that time he finds that there has been some increase in content of chlorine and total solids. As variable proportions of water are drawn from these two sources, the tap water used in our experiments will vary in composition between (or possibly above) the limits shown.

	Well 1	Well 2
Total solids	369.0	464.0
SiO_2	20.0	38.4
Fe_2O_3 Al_2O_3	3.2	5.6
Ca	49.2	53.7
Mg	12.1	15.2
SO_4	34.0	40.3
Cl	40.0	86.7
Total alkali	220.0	221.0
Free alkali	51.2	28.0
Acid hardness	169.0	193.0

Kepner (1914) and others have reported that "hard" wash water gives much higher yields of wet gluten and slightly higher yields of dry gluten than "soft" wash water. Yet most experts agree that distilled water can not be used.

Wood and Hardy (1909) believe that it is because of its carbonic acid content that distilled water disperses gluten. They believe gluten to be insoluble in pure water. If so, boiled distilled water would seem to be suitable for gluten washing.

Wood and Hardy used boiled distilled water in washing the glutens they used in their imbibition experiments. They noted, however, that the resultant glutens had physical properties different from those of glutens washed out with tap water. Upson and Calvin (1915) made similar observations.

There certainly is no conclusive evidence for the belief of Wood and Hardy that gluten is insoluble in boiled distilled water. It is highly improbable that the isoelectric points of the three constituent proteins are all exactly at neutrality. Combinations of anions or cations with these proteins very possibly exist in the flour. Such compounds may have appreciable solubility in water. For that matter the recent observation of Cohn (1922) indicates that even pure casein is slightly soluble at its isoelectric point. The same may be true of the gluten proteins.

That the character of the wash water is of great moment in washing gluten was recognized by LeClerc (1920) acting as referee for the Association of Official Agricultural Chemists. He and collaborators reported that washing gluten with distilled water containing 0.1% sodium chloride gave results comparable with the washings with local tap water.

In addition to producing differences in yields of gluten, different tap waters may produce differences in their physical character. Whymper (1920) has stated that soft alkaline waters destroy the springiness of gluten by preventing the coherence of its particles.

The advisability of replacing tap water with a more suitable wash water is closely connected with the hydrogen-ion concentration of the flour. Jessen-Hansen (1911) first reduced to an exact basis our knowledge of the hydrogen-ion concentration of flour and dough. He found that very acid old flours from which no gluten could be washed by the usual method, yield gluten if they are doughed with enough alkali to reduce the acidity suitably.

In addition to varying considerably in hydrogen-ion concentration, flours vary greatly in the kind and concentration of electrolytes, as shown by the variable ash content. Moreover, the amount and character of the inorganic constituents of a given flour are influenced by chlorine bleaching, for Bailey and Johnson (1924) have shown that the specific conductivity of the water extracts of flours is positively correlated with chlorine dosage.

Wood and Hardy (1909), Upson and Calvin (1915), and Lüers and Ostwald (1920) are of the opinion that gluten quality is controlled by the kind and concentration of anions and cations in the flour. The fact that a considerable proportion of inorganic constituents is present in washed-out gluten naturally suggests a relation between its physical properties and the variable nature and concentration of these electrolytes. The investigations of Gortner and Doherty (1918) led them to the conclusion that, apart from the influence of electrolytes, there are inherent physical properties of the gluten which vary from flour to flour and which are important in determining flour strength. Later, Sharp and Gortner (1923) advanced the view that glutenin is the protein responsible for inherent differences in gluten quality. Gortner has recently (1924) described in detail a method of evaluating glutenin quality by viscosity measurements. This method involves removal of water-soluble electrolytes from each of four suspensions of different concentrations of the flour. These suspensions are then brought to a pH of 3.0, the viscosity of each is determined, and the necessary calculations are carried out. However, there is no assurance that electrolytes which are not removed with water and which are either insoluble or are adsorbed by or chemically combined with the protein micellae near neutrality, come into action when the suspension is made so distinctly acid. It therefore seems likely that the question whether some other influence than the kind and concentration of anions and cations modifies gluten (or glutenin) quality is still debatable.

It was formerly considered that the glutenin-gliadin ratio was an important factor in determining gluten quality. The unreliability of the methods for determining glutenin and gliadin leaves this question open. Using an improved procedure, Sharp and Gortner (1923) concluded that the glutenin-gliadin ratio is too constant to have material influence on gluten quality. Calculation by the writers of the glutenin-gliadin ratios in the 11 flours which Sharp and Gortner analyzed gave values varying from a minimum of 0.88 (No. 1006) to a maximum of 1.18 (No. 1004).

Finally, in some flours gluten washing may be difficult on account of low protein content. When dispersion and loss of protein occurs in gluten washing, particularly in the early stage of the process, lack of quantity rather than lack of quality may cause the difficulty. Olsen (1912) has suggested that mixing such a flour with a flour of known high gluten content makes the estimation in such flour possible.

Summarizing the situation with regard to gluten washing, we may say that in spite of its defects it is proving of value to cereal chemists, primarily because it gives a partial measure of flour strength, giving in the one determination a rough index both to gluten quality and to gluten quantity.

The most evident defect in the present method appears to be the variable nature of the wash water employed. No one has yet established the relation between the electrolytes of the wash water, gluten washing, and gluten quality. In the light of the observations of different investigators presented, it seems likely that there is an optimum hydrogen-ion concentration and an optimum concentration of other ions for gluten washing. A further study of gluten washing with the object of controlling these as well as other factors which hitherto have not been considered might be profitable. We have therefore selected as our problem a careful study of the gluten washing procedure. An effort has been made to improve and standardize the method rather than to find new grounds for discarding it.

Preliminary Experiments

As already noted, gluten has been considered to be insoluble in distilled water free from carbon dioxide. In our first experiments glutens were washed from a commercial family patent flour with the local tap water, with boiled distilled water, and with a 0.1% solution of sodium chloride in boiled distilled water. At the same time the effect of prolonged washing was determined. Nitrogen determinations by the Kjeldahl-Gunning method were made on the dried glutens. Aside from variations in wash water and in length of the washing

period, the method of the Association of Official Agricultural Chemists was followed. The results are given in Table I.

TABLE I

EFFECT OF PROLONGED WASHING OF GLUTEN WITH TAP WATER, BOILED DISTILLED WATER, AND 0.1% NaCl

Expt. No.	I Wash water	II Washing period, min.	III Wet gluten, %	IV Dry gluten, %	V Moisture in moist gluten, %	VI Protein content of dry gluten, %	VII Gluten protein, % in flour
49	Tap water	25	35.60	10.96	69.2	78.8	8.64
50	" "	25	35.88	11.00	69.3	78.9	8.68
60	" "	35	34.28	10.48	69.4	82.4	8.64
59	" "	60	31.28	10.08	67.8	83.5	8.42
51	Distilled water	15	31.72	9.92	68.7	80.6	8.00
52	" "	15	32.12	9.84	69.4	82.5	8.12
53	" "	25	29.52	9.48	67.9	82.7	7.84
54	" "	25	28.60	9.00	68.5	84.5	7.60
55	" "	35	25.24	8.16	67.7	83.8	6.84
56	" "	60	19.16	6.76	64.7	86.4	5.84
77	0.1% Na Cl	15	33.36	10.48	68.6	82.4	8.64
78	" " "	15	34.88	10.56	69.7	81.8	8.64
79	" " "	25	33.44	10.24	69.4	83.8	8.58
80	" " "	25	33.24	10.36	68.8	83.4	8.64

Columns I to V of Table I are self-explanatory. Column VI records the protein content (N × 5.7) of the dry gluten. High values in this column are due to relatively greater removal of non-protein constituents. Column VII represents the percentage of gluten protein calculated to the flour basis. The values of Column VII are calculated from the formula:

$$\frac{\%\text{ of dry gluten} \times \%\text{ of protein in dry gluten}}{100} \quad \text{or} \quad \frac{\text{IV} \times \text{VI}}{100}$$

The results with tap water show that there is a progressive decrease in the non-nitrogenous constituents of the gluten with the prolongation of the washing period. At the same time there is only a slight decrease in gluten protein, which indicates that there is only slight dispersion of the proteins of this gluten by the local tap water. The wash water obtained after sixty minutes washing was turbid and gave a distinct test for starch. The figures in Column V suggest that the water-holding capacity of gluten is lessened by prolonged washing.

The results with freshly boiled distilled water prove that considerable dispersion occurs in the absence of electrolytes in the wash water. There was about the same loss from the 35th to the 60th minute as occurred from the 15th to the 25th minute. Here, too, there is some indication that with increasing proportion of protein in the gluten the water-holding capacity decreases, for No. 56 had the highest protein content and held the least water.

The results with 0.1% sodium chloride in freshly boiled distilled water are comparable with those obtained with tap water so far as protein dispersion and water-holding capacity are concerned. When salt solution was used the per cent of dry gluten was lower than when tap water was used (Nos. 79 and 80 compared with Nos. 49 and 50), indicating a more complete removal of non-protein constituents by the salt solution.

The physical properties of the glutens obtained by washing with tap water and with 0.1% sodium chloride solution were not distinctly different. The gluten obtained by washing with distilled water was less coherent and much difficulty was experienced in preventing mechanical loss during washing.

In the next study another family patent flour was employed. A stock sodium phosphate buffer solution of pH 7.6 was prepared. This had a concentration of 4.00% calculated as Na_2HPO_4. Dilutions to various concentrations were made as indicated in Table II. It was found that there was no notable change in pH as this solution was diluted with boiled distilled water. Boiled distilled water and tap water were also used for comparative purposes.

The time of washing was twenty-five minutes. The velocity of the stream was about 75 cc. per minute so that a total volume of about 2 liters was employed for each determination. No other step in the method was varied. The results follow in Table II.

TABLE II

EFFECT ON GLUTEN WASHING OF VARYING THE CONCENTRATION OF SALTS IN THE WASH WATER

I	II	III	IV	V	VI	VII
Expt. No.	Concentration, %	Wet gluten, %	Dry gluten, %	Moisture in wet gluten, %	Protein content of dry gluten, %	Gluten protein, % in flour
19	1.25*	31.80	10.68	66.4	75.7	8.08
20	1.25	32.16	10.92	66.0	73.2	8.00
21	0.50	33.44	10.36	69.1	79.1	8.20
22	0.50	33.64	10.32	69.3	78.3	8.08
23	0.25	34.28	10.56	69.2	77.6	8.20
24	0.25	34.64	10.44	70.0	78.9	8.24
25	0.10	35.04	10.80	69.2	78.1	8.44
26	0.10	35.25	10.84	69.3	79.2	8.48
27	0.01	34.60	11.08	68.0	77.3	8.56
28	0.01	33.24	10.80	67.5	78.1	8.44
15	0.00†	32.60	10.20	68.7	80.8	8.24
16	0.00†	32.44	10.04	69.0	80.9	8.12
17	0.05‡	34.36	10.76	68.7	78.1	8.40
18	0.05‡	34.16	10.68	68.7	80.2	8.56

*Nos. 19-28 were washed with a sodium phosphate solution of pH = 7.6 and of the indicated concentration. †Boiled distilled water. ‡Tap water.

The data in Table II indicate that sodium phosphate solutions of a concentration of 0.25% or more have considerable solvent effect on gluten protein. It is not improbable that globulin is removed by solu-

tions of such concentration. With 0.10% and 0.01% solutions, results comparable with those from the use of tap water were obtained. The glutens obtained with the lower concentrations of buffer solution had physical properties similar to those obtained with tap water.

Final Experiments

The flours employed in the subsequent work consisted for the most part of soft wheat flours which had been reported to offer some difficulty in gluten washing. Sodium phosphate solutions of 0.1% concentration and of hydrogen-ion concentrations corresponding to pH values of 4.4, 5.4, 5.8, 6.8, and 7.6 were employed both for doughing and washing. Tap water and 0.1% solution of calcium chloride (calculated to the anhydrous basis) and of sodium chloride were also employed. These last two solutions were tested colorometrically and found to be neutral.

The method of gluten washing employed in these final experiments follows: Weigh 25 grams of flour into a cup and add sufficient wash water (about 15 cc.) to make a firm dough ball. Work into a homogenous dough with a spatula, taking care that none of the material adheres to the cup. Cover the dough with wash water and allow to stand immersed for exactly one hour. Then remove the dough to the palm of one hand and knead gently in a stream of wash water, the temperature of which is about 20°. The stream should be regulated to deliver about 100 cc. per minute. In from seven to ten minutes the major portion of the starch will have been removed. This will be evident by a somewhat abrupt change in appearance of the wash water. Continue the washing for three minutes longer. The whole operation will usually require about twelve minutes and should be performed over a bolting-cloth sieve. Particles of gluten which are mechanically lost may be recovered by rolling the gluten ball over the sieve. Allow the gluten thus obtained to stand immersed in the wash water for about an hour, then press as dry as possible between the hands, place in a tared flat-bottomed dish, and weigh as moist gluten. Transfer to an oven, dry to constant weight at 100° (about 72 hours), cool, and weigh as dry gluten. The nature and preparation of the wash water will be described later.

The above method was followed in detail except that the nature of the wash water was varied. In most other respects it is similar to the method of the Association of Official Agricultural Chemists. It differs in the omission of the turbidity test for determining when washing is complete. As noted above, wash water remained slightly turbid even after an hour's washing. Preliminary investigation proved that drying was never complete after 24 hours at 100° and atmospheric

pressure. Seventy-two hours under these conditions was found necessary. Many procedures have been described for rapid drying of wet gluten which may be advantageous when speed is an important object, but none of these was tested in the present investigation.

The first flour employed was a commercial family patent. In addition to the solutions above described, 0.1% sodium phosphate buffer solutions of pH values 2.3, 2.8, and 3.5 were employed. These solutions produced such great dispersion of the gluten that it was entirely impossible to wash it out. The results are shown in Table III.

The values in Column VII indicate that between the ranges of hydrogen-ion concentration represented by pH values of 5.4 to 7.6 there is no great difference in protein dispersion with this flour. With a pH of 4.4 and with 0.1% calcium chloride solution there was marked loss of protein. The largest proportion of non-protein constituents was removed by the calcium chloride solution.

Similar observations on a club wheat patent flour are assembled in Table IV. It is noted that the dispersion of proteins decreased from a maximum when the buffer solution of hydrogen-ion concentration indicated by pH 4.4 was used to a minimum at pH 6.8. Calcium chloride solution again produced high dispersion of proteins and relatively high protein content of the dry gluten. Tap water and sodium chloride both produced greater dispersion of protein than the optimal buffer solution.

In general the results with the other seven flours shown in Tables V to XI are parallel to those obtained with the first two and hence need not be described in detail. Individual determinations are given in order that the probable error may be appreciated.

TABLE III

EFFECT OF VARIOUS SALTS AND OF 0.1% SODIUM PHOSPHATE BUFFER SOLUTIONS OF VARIOUS pH VALUES ON GLUTEN WASHING

Commercial family patent

I Expt. No.	II pH	III Wet gluten, %	IV Dry gluten, %	V Moisture in wet gluten, %	VI Protein content of dry gluten, %	VII Gluten protein, % in flour
81	2.3	Impossible to wash		....	....	....
82	2.8	" " "		....	....	....
83	3.5	" " "		....	....	....
87	4.4	33.68	9.60	71.5	81.8	7.96
88	4.4	34.28	10.12	70.5	82.7	8.04
231	5.4	36.80	11.20	69.6	76.8	8.60
232	5.4	36.00	10.88	69.8	79.8	8.68
233	5.8	36.32	11.12	69.4	78.4	8.72
234	5.8	36.72	11.12	69.7	76.6	8.52
89	6.8	35.92	11.32	68.5	76.7	8.68
90	6.8	35.48	10.96	69.1	78.8	8.64
91	7.6	36.64	11.04	69.8	76.4	8.44
92	7.6	36.98	11.12	69.9	77.7	8.64
93	*	35.64	9.92	72.2	83.1	8.24
94	*	36.52	9.84	73.0	83.4	8.20
77	†	33.36	10.48	68.6	82.4	8.64
78	†	34.88	10.58	69.7	81.6	8.64
49	‡	35.60	10.96	69.2	78.8	8.64
50	‡	35.88	11.00	69.3	78.9	8.68

*0.1% $CaCl_2$ solution.
†0.1% NaCl solution.
‡Tap water.

TABLE IV

EFFECT OF VARIOUS SALTS AND OF 0.1% SODIUM PHOSPHATE BUFFER SOLUTIONS OF VARIOUS pH VALUES ON GLUTEN WASHING

Club wheat patent

I Expt. No.	II pH	III Wet gluten, %	IV Dry gluten, %	V Moisture in wet gluten, %	VI Protein content of dry gluten, %	VII Gluten protein, % in flour
107	4.4	25.40	7.76	69.4	72.2	5.60
108	4.4	26.60	8.12	69.4	69.7	5.68
109	5.4	26.64	8.76	67.1	69.4	6.08
110	5.4	27.48	8.90	67.6	69.7	6.20
111	5.8	27.88	9.04	67.6	69.0	6.24
112	5.8	27.84	9.16	67.1	68.1	6.24
113	6.8	27.36	8.68	68.3	72.4	6.28
114	6.8	26.52	8.48	68.0	74.0	6.28
115	7.6	27.32	8.80	67.8	71.4	6.28
116	7.6	26.08	8.44	67.6	73.0	6.16
105	*	26.32	7.72	70.7	72.5	5.60
106	*	25.28	7.56	70.1	75.1	5.68
103	†	27.44	8.72	68.2	70.6	6.16
104	†	26.92	8.72	67.6	69.2	6.04
101	‡	24.84	8.28	66.7	74.4	6.16
102	‡	26.52	8.80	66.9	70.0	6.16

*0.1% $CaCl_2$ solution.
†0.1% NaCl solution.
‡Tap water.

TABLE V

EFFECT OF VARIOUS SALTS AND OF 0.1% SODIUM PHOSPHATE BUFFER SOLUTIONS OF VARIOUS pH VALUES ON GLUTEN WASHING

Soft red winter wheat flour from Maryland

I Expt. No.	II pH	III Wet gluten, %	IV Dry gluten, %	V Moisture in wet gluten, %	VI Protein content of dry gluten, %	VII Gluten protein, % in flour
137	4.4	Impossible to wash		....	....	....
138	4.4	" " "		....	....	....
139	5.4	30.56	8.80	71.2	77.7	6.84
140	5.4	30.12	8.68	71.2	77.9	6.76
141	5.8	28.64	8.76	69.4	77.2	6.76
142	5.8	29.88	8.88	70.2	78.8	7.00
143	6.8	28.24	8.96	68.3	77.7	6.96
144	6.8	28.72	9.16	68.1	76.0	6.96
145	7.6	29.40	8.92	69.7	76.7	6.84
146	7.6	28.64	8.96	68.7	76.3	6.84
147	*	30.64	8.36	72.7	80.4	6.72
148	*	30.24	8.44	72.1	81.5	6.88
127	*	25.76	8.20	68.1	77.9	6.40
128	*	26.12	8.40	67.9	76.2	6.40
135	†	30.08	9.08	69.8	76.2	6.92
136	†	28.80	8.92	69.1	76.2	6.80
125	†	29.16	8.96	69.3	74.3	6.66
126	†	29.04	9.08	68.7	74.0	6.72
129	‡	28.84	9.16	68.3	76.4	7.00
130	‡	28.96	9.12	68.5	75.9	6.92

*0.1% $CaCl_2$ solution.
†0.1% NaCl solution.
‡Tap water.

TABLE VI

EFFECT OF VARIOUS SALTS AND OF 0.1% SODIUM PHOSPHATE BUFFER SOLUTIONS OF VARIOUS pH VALUES ON GLUTEN WASHING

Soft white winter wheat flour from eastern Washington

I Expt. No.	II pH	III Wet gluten, %	IV Dry gluten, %	V Moisture in wet gluten, %	VI Protein content of dry gluten, %	VII Gluten protein, % in flour
155	4.4	.....	7.72	....	74.7	5.72
156	4.4	.....	7.60	....	75.2	5.76
157	5.4	25.45	8.28	67.5	73.3	6.08
158	5.4	25.88	8.12	68.6	75.8	6.16
159	5.8	27.60	8.96	67.5	69.4	6.20
160	5.8	27.72	8.64	68.8	73.3	6.32
161	6.8	26.00	8.52	67.2	74.9	6.40
162	6.8	26.24	8.80	66.5	74.4	6.56
163	7.6	26.04	8.24	68.3	72.4	5.96
164	7.6	26.24	8.32	68.3	73.9	6.26
167	*	29.88	8.64	71.1	71.3	6.16
168	*	29.04	8.40	71.1	72.9	6.12
165	†	26.08	8.44	67.6	73.5	6.20
166	†	25.96	8.40	67.6	75.0	6.28
153	‡	24.72	8.36	66.2	73.6	6.16
154	‡	25.56	8.52	66.7	74.0	6.32

*0.1% $CaCl_2$ solution.
†0.1% NaCl solution.
‡Tap water.

TABLE VII

EFFECT OF VARIOUS SALTS AND OF 0.1% SODIUM PHOSPHATE BUFFER SOLUTIONS OF VARIOUS pH VALUES ON GLUTEN WASHING

Commercial 20% high grade patent from Tennessee

I Expt. No.	II pH	III Wet gluten, %	IV Dry gluten, %	V Moisture in wet gluten, %	VI Protein content of dry gluten, %	VII Gluten protein, % in flour
169	4.4	Impossible to wash		....	....	....
170	4.4	" " "		....	....	....
171	5.4	33.04	9.24	72.0	80.5	7.44
172	5.4	32.84	9.36	71.5	79.5	7.44
173	5.8	33.44	10.24	69.4	76.6	7.84
174	5.8	33.16	10.28	69.0	76.1	7.84
175	6.8	32.20	10.40	67.7	73.8	7.68
176	6.8	32.40	10.24	68.4	77.3	7.92
177	7.6	32.76	10.88	66.8	71.3	7.76
178	7.6	31.48	10.40	67.0	74.3	7.76
167	*	.....	8.32	....	83.7	6.96
168	*	.....	9.04	....	79.2	7.16
179	†	32.00	9.00	71.9	82.7	7.44
180	†	31.88	9.00	71.8	82.7	7.44
181	‡	31.84	10.24	67.8	76.2	7.80
182	‡	31.60	10.32	67.3	75.6	7.80

*0.1% $CaCl_2$ solution.
†0.1% NaCl solution.
‡Tap water.

TABLE VIII

EFFECT OF VARIOUS SALTS AND OF 0.1% SODIUM PHOSPHATE BUFFER SOLUTIONS OF VARIOUS pH VALUES ON GLUTEN WASHING

10% Low grade flour from Tennessee

I Expt. No.	II pH	III Wet gluten, %	IV Dry gluten, %	V Moisture in wet gluten, %	VI Protein content of dry gluten, %	VII Gluten protein, % in flour
183	4.4	35.52	11.28	68.2	79.3	8.96
184	4.4	36.12	11.52	68.1	75.7	8.72
185	5.4	36.32	11.72	67.7	75.7	8.88
186	5.4	36.56	11.76	67.8	75.5	8.88
187	5.8	37.42	11.80	68.4	76.6	9.04
188	5.8	37.16	11.76	68.3	76.9	9.04
189	6.8	37.76	11.84	68.6	77.7	9.20
190	6.8	37.88	11.68	69.2	77.1	9.00
191	7.6	38.00	11.52	69.7	78.1	9.00
192	7.6	36.76	11.44	68.9	78.7	9.00
197	*	38.08	10.12	73.4	82.2	8.32
198	*	37.04	10.04	72.9	84.5	8.48
193	†	38.48	11.72	69.5	76.5	8.96
194	†	38.36	11.80	69.3	75.6	8.92
195	‡	37.84	11.84	68.7	76.0	9.00
196	‡	36.48	11.48	68.5	77.7	8.92

*0.1% $CaCl_2$ solution.
†0.1% NaCl solution.
‡Tap water.

TABLE IX

EFFECT OF VARIOUS SALTS AND OF 0.1% SODIUM PHOSPHATE BUFFER SOLUTIONS OF VARIOUS pH VALUES ON GLUTEN WASHING

30% Cut-off flour (with low grade removed) from Tennessee

I Expt. No.	II pH	III Wet gluten, %	IV Dry gluten, %	V Moisture in wet gluten, %	VI Protein content of dry gluten, %	VII Gluten protein, % in flour
199	4.4	.....	11.00	....	78.2	8.60
200	4.4	.....	11.08	....	77.6	8.60
201	5.4	36.72	11.44	68.8	78.0	8.92
202	5.4	36.80	11.20	69.6	79.5	8.92
203	5.8	38.84	11.84	69.5	76.3	9.04
204	5.8	39.40	12.00	69.5	75.4	9.04
205	6.8	38.64	11.92	69.1	76.8	9.16
206	6.8	38.64	12.24	68.3	74.9	9.16
207	7.6	38.52	11.68	69.7	76.4	8.92
208	7.6	38.92	11.76	69.8	76.5	9.00
211	*	39.16	11.08	71.7	77.1	8.56
212	*	37.64	10.24	72.8	80.1	8.20
209	†	39.04	11.56	70.4	75.1	8.68
210	†	38.84	11.48	70.5	76.3	8.76
213	‡	37.92	11.84	68.8	76.7	9.08
214	‡	38.48	12.04	68.7	75.1	9.04

*0.1% $CaCl_2$ solution.
†0.1% NaCl solution.
‡Tap water.

TABLE X

EFFECT OF VARIOUS SALTS AND OF 0.1% SODIUM PHOSPHATE BUFFER SOLUTIONS OF VARIOUS pH VALUES ON GLUTEN WASHING

100% Straight flour from Tennessee

I Expt. No.	II pH	III Wet gluten, %	IV Dry gluten, %	V Moisture in wet gluten, %	VI Protein content of dry gluten, %	VII Gluten protein, % in flour
215	4.4	.....	10.68	....	76.4	8.16
216	4.4	.....	10.52	....	76.4	8.04
217	5.4	34.52	10.52	69.5	79.1	8.32
218	5.4	35.32	10.84	69.3	76.0	8.24
219	5.8	35.48	10.76	69.9	77.7	8.36
220	5.8	34.64	10.52	69.7	79.5	8.36
221	6.8	36.12	11.44	68.3	73.1	8.36
222	6.8	35.84	11.40	68.2	74.0	8.44
223	7.6	35.48	10.80	69.6	78.1	8.44
224	7.6	35.00	11.00	68.6	75.7	8.32
227	*	35.56	9.92	72.1	79.5	7.88
228	*	35.84	9.56	73.3	82.0	7.84
225	†	35.04	10.72	69.3	76.9	8.24
226	†	35.76	10.72	70.0	76.9	8.24
229	‡	35.08	11.00	68.6	75.3	8.28
230	‡	34.56	10.68	69.1	78.3	8.36

*0.1% $CaCl_2$ solution.
†0.1% NaCl solution.
‡Tap water.

TABLE XI

EFFECT OF VARIOUS SALTS AND OF 0.1% SODIUM PHOSPHATE BUFFER SOLUTIONS OF VARIOUS pH VALUES ON GLUTEN WASHING

Eastern soft red winter wheat flour

I Expt. No.	II pH	III Wet gluten, %	IV Dry gluten, %	V Moisture in wet gluten, %	VI Protein content of dry gluten, %	VII Gluten protein, % in flour
235	4.4	.....	10.60	....	74.4	7.88
236	4.4	.....	10.80	....	74.4	8.04
237	5.4	36.28	10.52	71.0	79.4	8.36
238	5.4	37.00	10.80	70.8	77.8	8.40
239	5.8	34.96	10.48	70.0	79.7	8.36
240	5.8	36.88	10.72	70.9	78.0	8.36
241	6.8	35.08	10.80	69.2	78.5	8.48
242	6.8	34.92	11.12	68.2	75.9	8.44
243	7.6	35.68	11.40	68.6	73.7	8.40
244	7.6	35.52	11.32	68.2	74.2	8.40
245	*	38.04	10.52	72.3	76.4	8.04
246	*	38.04	10.48	72.5	78.2	8.20
247	†	37.28	11.24	69.8	75.1	8.44
248	†	37.04	11.16	69.8	75.7	8.44
249	‡	34.64	10.96	68.6	77.0	8.44
250	‡	36.20	11.48	68.3	73.9	8.48

*0.1% $CaCl_2$ solution.
†0.1% NaCl solution.
‡Tap water.

The water contents of the different glutens are collected in Table XII. Averages of duplicate determinations are given. While somewhat variable values were found in glutens from different flours washed out with the more acid and alkaline buffer solution, the buffer solution nearest neutrality produced glutens with almost identical water content for all the flours. The water contained in these glutens is nearly identical with the water contained in the tap water glutens.

Most striking is the high water content of the calcium chloride glutens. In eight of the flours the water content was greatest in these glutens. The missing values in this table are explained by the fact that some glutens (particularly those which were washed out with the most acid buffer solution) dispersed to such an extent during the one-hour period following washing that it was impossible to remove the excess moisture by rolling the gluten ball in the hands. The flour of Table VII produced gluten of such a character. It is interesting to note that the calcium chloride gluten from this flour was the only one which dispersed to such an extent that drying in the hands was impossible. Of all the sodium chloride glutens, that from this flour contained most water. Also, of all the glutens washed with pH 5.4 buffer solution, that from this flour had the highest water content. On the other hand, of all the glutens washed with pH 7.6 buffer solution, that from this flour contained least water.

TABLE XII

MOISTURE CONTENT OF GLUTENS FROM VARIOUS FLOURS

Flour used	Solutions employed in washing: 0.1% Buffer solution of indicated pH 4.4	5.4	5.8	6.8	7.6	0.1% $CaCl_2$	0.1% NaCl	tap water
Commercial family patent	71.0	69.7	69.6	68.8	69.8	72.6	69.1	69.3
Club wheat patent	69.4	67.4	67.4	68.2	67.7	70.4	67.9	66.6
Soft red winter wheat flour from Maryland	....	71.2	69.8	68.2	69.2	72.4	69.5	68.4
Soft white winter wheat flour from eastern Washington	....	68.1	68.1	66.9	68.3	71.1	67.6	66.4
Commercial 20% high grade patent flour from Tennessee	....	71.8	69.2	68.1	66.9	....	71.9	67.6
10% low grade flour from Tennessee	68.2	67.8	68.4	68.9	69.3	73.2	69.4	68.6
30% Cut-off flour (with low grade removed) from Tenn.	....	69.2	69.5	68.7	69.8	72.3	70.5	68.8
100% straight flour from Tennessee	....	69.4	69.8	68.3	69.1	72.7	69.7	68.9
Eastern soft red winter wheat flour	....	70.9	70.5	68.7	68.4	72.4	69.8	68.4

Tables III-XI furnish data from which the relation between water content and protein content of gluten may be shown. There are 67 pairs of duplicate analyses in these tables. Comparing these duplicates, 29 pairs showed lack of agreement in water content of 0.5% or more. Of these 29, 20 showed differences in the same direction in protein percentage, 7 showed differences in the opposite direction, and 2 agreed. The high per cent of water in the calcium chloride glutens may also be related to their high protein percentage. On the other hand, it will be recalled that when glutens were subject to prolonged washing (Table I) their water content did not increase as did their protein percentage but on the contrary seemed to decrease.

We can therefore reach no final conclusion on the relation between protein content and water content. Therefore any conclusion as to gluten quality which is based on imbibition determination without taking into account differences in protein content is open to question. Thus in one of the earlier studies of Gortner and Sharp (1922), the percentage of protein in four glutens whose imbibition properties were compared, ranged from 56.26 to 79.42%.

The glutens washed with the pH 6.8 buffer solution were of variable quality as evidenced by differences in coherence and tenacity. The same is true of those washed out with tap water. However, those washed out with 0.1% $CaCl_2$ solution were all similar in quality. They were almost uniformly flabby and incoherent.

These different effects on water-holding capacity and quality produced in glutens by the different wash waters can not be explained entirely by differences in molar concentration of the wash waters. The data in Table II show that ranges in concentration of sodium phosphate from 0.01% to 0.50% produced no great changes in water-holding capacity. The corresponding range in approximate molar concentration (of Na_2HPO_4) is from M/1400 to M/28. A 0.1% solution of calcium chloride is M/110, while a 0.1% solution of sodium chloride is M/58. Neither can it be explained by differences in hydrogen-ion concentration, for the sodium chloride and calcium chloride solutions were neutral.

In order to investigate the effect of more dilute calcium and sodium chloride solution, a 0.01% solution of each was employed on one flour. These results are found in Table V. There was so much dispersion of protein and consequent difficulty in washing by these two solutions, especially by the calcium chloride solution, that no further attempt was made to use them. Less water was contained in the glutens washed out with 0.01% calcium chloride than in the glutens washed

out by 0.10% calcium chloride. The data are too meager to warrant further discussion. That the calcium ion has a pronounced direct or indirect effect on the physical properties of gluten can scarcely be denied.

The local tap water has a concentration of 0.05% total solids. Its content of calcium salts is considerable. Yet it exerts no such prejudicial action on gluten quality as does a pure calcium chloride solution of 0.10% concentration. Possibly the lower percentage of calcium is the explaining factor. It seems more likely that there is something akin to an antagonistic action of the other ions of tap water. It does not seem entirely improbable, just as there is a balanced salt solution adapted to many vital processes so there may also exist a balanced electrolyte mixture most suitable for development of optimum gluten quality.

A summary of the yields of gluten protein obtained from the various flours is shown in Table XIII. These figures are averages of the duplicate determinations shown in the earlier tables. The data of Table XIII have been placed in more readily interpretable form in Table XIV. For each flour the maximal yield of gluten protein has been represented as 100 in the latter table. The other values have been calculated to this base. Thus with the first flour listed maximal gluten protein yields were obtained with pH 6.8 buffer solution and with tap water. In Table XIV this is indicated by the figure 100 for each of these wash waters. With the same flour, 0.1% calcium chloride solution yielded 94.9% of this maximal gluten protein value.

It will be noted in Table XIV that maximal yield (which means minimal dispersion) of gluten protein was obtained at pH 6.8 in eight of the nine flours. In three of these eight flours the same value was obtained with tap water. In one case the maximal result was obtained with the buffer solution of pH 5.8.

The averages of these values are shown at the bottom of Table XIV and need little comment. In general the observation was made that difficulty in washing is least where there is least dispersion of protein. With some flours much less difficulty in washing was experienced with the buffer solution of pH 6.8 than with tap water. The observation that minimum dispersion of gluten protein occurs at a pH of 6.8 or thereabouts is in harmony with the observations of Lüers (1919) who found the isoelectric point of gliadin to be near the hydrogen-ion concentration corresponding to a pH value of 6.7.

TABLE XIII

PERCENTAGE OF GLUTEN PROTEIN (CALCULATED TO THE FLOUR BASIS) OBTAINED FROM VARIOUS FLOURS

Flour used	Solutions employed in washing: 0.1% Sodium phosphate solution of indicated pH 4.4	5.4	5.8	6.8	7.6	0.1% $CaCl_2$	0.1% NaCl	tap water
Commercial family patent	8.00	8.64	8.62	8.66	8.54	8.22	8.64	8.66
Club wheat patent	5.64	6.14	6.24	6.28	6.22	5.64	6.10	6.16
Soft red winter wheat flour from Maryland	....	6.80	6.88	6.96	6.84	6.80	6.86	6.96
Soft white winter wheat flour from eastern Washington	5.74	6.12	6.26	6.48	6.11	6.14	6.24	6.24
Commercial 20% high grade patent flour from Tennessee	....	7.44	7.84	7.80	7.76	7.06	7.44	7.80
10% low grade flour from Tennessee	8.84	8.88	9.04	9.10	9.00	8.40	8.94	8.96
30% Cut-off flour (with low grade removed) from Tenn.	8.60	8.92	9.04	9.16	8.96	8.38	8.72	9.06
10% straight flour from Tennessee	8.10	8.28	8.36	8.40	8.38	7.86	8.24	8.32
Eastern soft red winter wheat flour	7.96	8.38	8.36	8.46	8.40	8.12	8.44	8.46

TABLE XIV

DISPERSION OF GLUTEN PROTEIN BY VARIOUS SOLVENTS. MAXIMUM YIELD OF PROTEINS FOR EACH FLOUR = 100%

Flour used	Solutions employed in washing: 0.1% Sodium phosphate solution of indicated pH 4.4	5.4	5.8	6.8	7.6	0.1% $CaCl_2$	0.1% NaCl	tap water
Commercial family patent	92.4	99.8	99.6	100.0	98.6	94.9	99.8	100.0
Club wheat patent	89.8	97.8	99.4	100.0	99.0	89.8	97.1	98.1
Soft red winter wheat flour from Maryland	....	97.7	98.9	100.0	98.3	97.7	98.6	100.0
Soft white winter wheat flour from eastern Washington	88.6	94.5	96.6	100.0	94.3	94.8	96.3	96.3
Commercial 20% high grade patent flour from Tennessee	....	94.9	100.0	99.5	99.0	90.1	94.9	99.5
10% low grade flour from Tennessee	97.2	97.6	99.4	100.0	98.9	92.3	98.2	98.5
30% Cut-off flour (with low grade removed) from Tenn.	93.9	97.4	98.7	100.0	97.8	91.5	95.2	98.9
100% straight flour from Tennessee	96.4	98.6	99.5	100.0	99.7	93.6	98.1	99.1
Eastern soft red winter wheat flour	94.1	99.1	98.8	100.0	99.3	96.0	99.8	100.0
Average	93.2	97.5	99.0	100.0	98.3	93.4	97.5	98.9

TABLE XV

RELATION OF TOTAL FLOUR PROTEIN TO DRY GLUTEN AND TO GLUTEN PROTEIN

Flour used	Total protein (N × 5.7)	Dry gluten, %	Gluten protein obtained with buffer solution (pH = 6.8), % in flour	% of total protein which appeared in gluten
Commercial family patent	10.09	11.14	8.66	85.8
Club wheat patent	7.50	8.58	6.28	83.7
Soft red winter wheat flour from Maryland	8.20	9.06	6.96	84.9
Soft white winter wheat flour from eastern Washington	7.52	8.66	6.48	86.2
Commercial 20% high grade patent flour from Tennessee	9.31	10.32	7.80	83.8
10% low grade flour from Tennessee	11.29	11.76	9.10	80.6
30% Cut-off flour (with low grade removed) from Tennessee	10.55	12.08	9.16	86.8
100% Straight flour from Tennessee	9.66	11.42	8.40	87.0
Eastern soft red winter wheat flour	10.01	10.96	8.46	84.5
Average	.....	.....	....	84.8

The relation of the total protein content of the flour to the percentages of dry gluten and of gluten protein is shown in Table XV. For the nine flours examined the yields of dry gluten exceeded the percentages of total protein by about one-tenth. If total protein is calculated with the factor 6.25, the two values check closely. The per cent of total protein of the flour which appeared in the gluten ranged from 80.6 to 87.0, averaging 84.8. This does not agree closely with the value of 74.6% found by Olsen (1912) as the average of twenty-four flours. Olsen also reported that one-fourth of the flours he examined had glutens which scattered badly. Eight of the nine flours we examined had been reported to offer some difficulty to gluten washing. None of these flours yielded as low a ratio of gluten protein to total protein as the average ratio which Olsen obtained. This also applies to the results obtained with tap water. Possibly the tap water which Olsen employed differs sufficiently from our tap water to explain the difference.

Naturally the question now arises whether or not the substitution of a standard wash water for tap water in gluten washing is advisable. It must be admitted that in our experiments the quality and yield of glutens obtained with the nearly neutral phosphate buffer solution checked closely with the quality and yield of glutens obtained with our local tap water. On the other hand, our experiments have fully demonstrated that glutens washed with distilled water differ in quantity and quality from those prepared with our tap water or with our neutral buffer solution. One has only to recall that in many regions of the United States the tap water closely approaches distilled water in nature, to appreciate the significance of this. The apparent specific effect on gluten quality of a pure calcium chloride solution is also of significance when one considers the great variation in both absolute and relative concentration of calcium salts in potable waters. Finally there is indirect evidence that the tap water employed in these experiments is especially effective in preventing gluten dispersion. As pointed out above, comparison of our results with those of Olsen's reveal differences which are difficult to explain on any other basis.

Believing that we have presented a sound case for the substitution of standard wash water for tap water, the selection of such a wash water remains to be considered. While our experiments have by no means exhausted the possibilities, certain fundamental facts have been established. The wash water should be practically neutral. It should contain approximately 0.10% solids altho slight variations in either direction do not seem to be significant. Calcium chloride was found to be entirely unsuitable. Sodium chloride in some cases was distinctly

inferior to our tap water. Neutral sodium phosphate was wholly satisfactory, giving in four of the nine cases as good results as tap water and in the other cases better results than tap water.

This wash water is especially advantageous in that it possesses a high buffer value, thus minimizing the effect on gluten washing of the high hydrogen-ion concentration which characterizes old flours. This masking of flour acidity might be raised as an objection to the method. Yet one should not expect to gain an insight into the hydrogen-ion concentration from the gluten washing process. Gluten washing should be carried out to determine gluten quantity and quality, and as pointed out, Jessen-Hansen has shown that with proper adjustment of the hydrogen-ion concentration the native quality of the gluten is restored.

The preparation of such a buffer solution offers no difficulties to laboratories equipped to determine hydrogen-ion concentration, either colorometrically or potentiometrically. The practice which has been followed has been to make up a 4% solution of disodium phosphate (anhydrous basis). Monosodium phosphate solution of the same concentration is added until a pH of 6.8 is reached. Slight variations in concentration were found to be without effect. It is believed that the hydrogen-ion concentration can be regulated with sufficient accuracy colorometrically. The solution should give the characteristic purple with brom-cresol-purple and should not give a distinct red with phenol-red. Dilution of the 4% stock solution with unboiled distilled water to 0.1% concentration does not significantly modify the hydrogen-ion concentration. A siphon and screw clamp permit use of the diluted stock solution at the proper velocity. About one liter is sufficient for a determination.

Summary

1. The effect of prolonging the gluten washing period when the tap water at Stanford University was used, was a continuous decrease in the non-nitrogenous constituents of the gluten. At the same time there was only a slight loss of the nitrogenous constituents.

2. Prolonged washing of gluten with boiled distilled water resulted in considerable dispersion of protein.

3. Gluten obtained with tap water was of better quality than gluten obtained with boiled distilled water.

4. Considerable variations in concentration of a sodium phosphate solution (pH 7.6) did not greatly influence gluten quality or yield.

5. Glutens obtained from different flours with a 0.1% neutral calcium chloride solution were all of poor quality, high water contents, and low non-protein contents.

6. Gluten washing with sodium phosphate buffer solutions of various hydrogen-ion concentrations demonstrated that there is minimum protein dispersion near neutrality.

7. Gluten washing with approximately neutral 0.1% sodium phosphate, 0.1% calcium chloride, and with tap water proved that the sodium phosphate solution was most effective in preventing gluten dispersion.

8. The importance of adopting this sodium phosphate solution as a standard wash water is pointed out. A simple method for preparing it is described.

9. A modified method for gluten washing is presented.

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GLUTEN QUALITY

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(Read at the Convention, June 10, 1924)

Wheat contains approximately only 10 per cent gluten, yet it is generally agreed that its quality very largely depends on the amount and quality of this ingredient. As cereal chemists, we are well satisfied with the present methods of determining the amount of gluten in wheat or flour. The protein test has become almost universal, and while it may be open to the objection that other proteins, aside from gluten, are included, these are of relatively small amount and constant.

Many chemists use the wet and dry gluten test with very satisfactory results. However, it is difficult to standardize conditions so that the latter method will generally give uniform results. It has the advantage, however, that we actually separate and have before us the particular gluten that we are determining. Many flour chemists are now using both of these methods and getting accurate and reliable results. The great difficulty is that they have not learned to talk in a universal language. Each thinks that he is right and the other fellow is wrong. Our company has six laboratories, some more than 1000 miles apart, and under widely different conditions, which check wet glutens to about one per cent. This is equivalent to 0.3 per cent protein, which I am sure all will agree is very accurate.

The individual working with glutens soon becomes aware that some glutens feel very different from others, and if they are observing they soon learn that each kind of wheat has a certain character of gluten. Further, it is also observed that growing conditions and soundness affect the character of the wheat.

From a practical standpoint the above conditions, together with the milling and strength, establish the value of a flour and its suitability for a certain purpose. To be more specific as to the character of glutens, we will describe the quality of some of the most important kinds.

Spring Wheat, Marquis

The gluten from this wheat washes out granular, short, and tough. A short gluten is one that can not be stretched out much and that breaks off sharp instead of stretching.

Winter Wheat, Turkey Red

The gluten is smooth, elastic, and has various degrees of toughness.

White Wheat, Bluestem and Baart

These wheats have a smooth, soft, elastic, and not very tough gluten.

Club Wheat

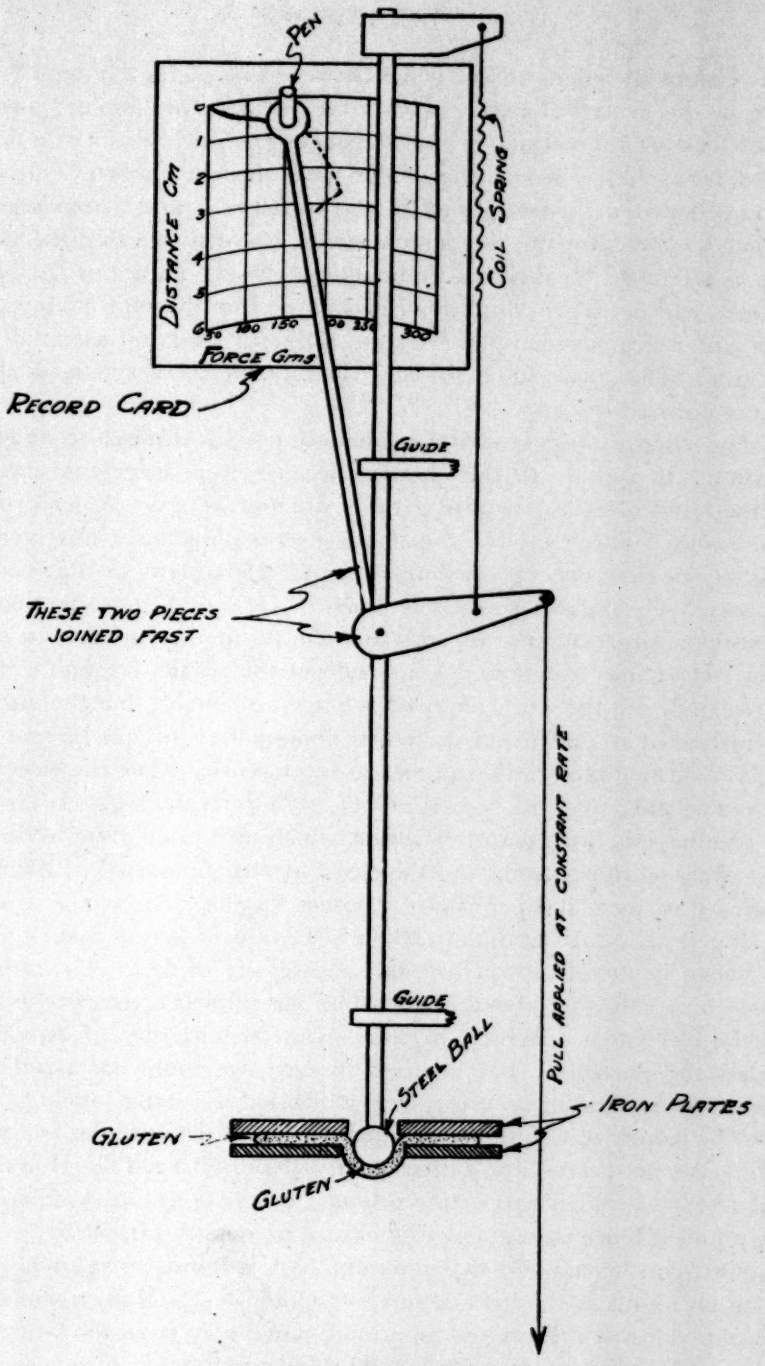
Typical wheats of this variety have a short, soft, and weak gluten; unusual samples are very soft and sticky.

Damaged wheat has a softened gluten, in extreme cases, soft and sticky. Wheat grown under unusual conditions usually has a softer gluten than No. 1. For instance, very dry weather which produces pinched and light weight wheat also produces a large percentage of gluten but of a soft quality. Very favorable growing conditions, such as plenty of moisture, result in a short gluten. If only sufficient to produce heavy wheat but of good strength, the quality will not be impaired and the shortening will give a partial spring wheat character.

The quality of a gluten can be well expressed by the following factors: the force required to stretch it, the distance through which it can be stretched before breaking, and whether it breaks sharply or gradually; and physical characteristics, such as granular, soft, and sticky, which are observed while washing.

Several devices have been constructed for making physical measurements on glutens, some of which are quite ingenious and useful. We have used one of home manufacture which consisted of two flat surfaces exactly parallel, the upper surface so arranged that it was free to move up and down through guides, and with a table at the top for supporting a weight. A micrometer was attached to the movable plate so that the distances between the fixed and movable plates could be accurately measured. After washing, the gluten was placed between the two plates and it was then possible to make measurements including resistance, extensibility, and time. On removing the weight, a second measurement indicates the "spring" of the gluten, which is much the same as the shortness of a spring wheat gluten. We were not satisfied with the results from this apparatus. It required too much time to use it and it was more practical to train workers to express the same characteristics descriptively. We believe that with certain refinements the method could be made very useful.

Mr. James, in charge of the Sperry laboratory at Spokane, has devised a most ingenious apparatus for testing glutens. This is shown diagrammatically in the figure. At one operation it makes a curve showing the resistance to stretching, the distance that a gluten can be stretched before breaking, and the character of the break, whether gradual or sharp. It has the advantage that the test can be made quickly and requires but little work. The operation is as follows: After washing the gluten it is allowed to stand in water until several samples are ready to test. Then it is put in a clamp which cuts out a pellet about one inch in diameter and $\frac{1}{8}$ inch thick, and at the same



GLUTEN TESTER

time clamps the edges of this pellet between two plates having a $\frac{3}{4}$-inch hole in the center of each. This leaves a piece of gluten $\frac{3}{4}$ inch in diameter and $\frac{1}{8}$ inch thick held at the edges on which we exert a downward force with a round faced plunger $\frac{1}{2}$ inch in diameter. It is so arranged that a card is placed in the instrument and a movable pen makes a curve showing the force exerted, the distance that the gluten can be stretched through before breaking, and the nature of the break. Results can be closely duplicated and agree thoroly with baking tests. The difference between glutens from different kinds of wheat is very striking. The apparatus is particularly valuable for very strong spring wheat flours.

The viscosity test is used considerably now as a measure of gluten quality. It appeals to the chemist because it is largely a chemical method and does not require a great amount of time to perform the operations. This method is open to several objections, however. It can be carried out by washing out the electrolyte or determining directly. The former method is much more logical and scientific, but requires a large amount of work and the results are difficult to duplicate. If the electrolyte is not washed out the results are more readily duplicated, and the work required is not unreasonable, but the viscosity as measured is due to an unknown combination of gluten and electrolyte. The gluten causes an increase in viscosity while the electrolyte is a repellant.

We have in mind a certain wheat which unwashed gives a viscosity test of 50. After washing out the electrolyte this is raised to 106, or an increase of over 100 per cent. Another similar flour which from all baking tests would be judged about 10 per cent better than the first, owing to better gluten quality, had a viscosity of 112. The point we wish to make is that gluten quality does not influence viscosity as much as the electrolyte. Hence, in comparing the viscosity of two flours, unless the electrolyte was identical in each, we could not assume that the viscosity readings were a true indication of gluten quality.

The baking test shows gluten quality, but it also includes everything else. We need a test for gluten quality alone. It need not be a chemical test. Chemical properties produce certain physical results. We may find it more convenient to measure its quality physically.

Our conclusions are that now our best judgment of gluten quality is to be found in the baking and wet gluten test. Many chemists are getting valuable information in an individual way from the latter. Our greatest need now is some method whereby we can express our results in accurate mathematical terms that can be standardized for general use.

FORMULAS AND METHOD OF PROCEDURE FOR EXPERIMENTAL BAKING TESTS

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(Read at the Convention, June 11, 1924)

Bringing this subject of experimental or laboratory baking tests before the association may result in differences of opinion and precipitate a discussion beside which the present heated argument between the modernists and the fundamentalists will pale into insignificance. However, this paper has been prepared only after the collection of considerable data and the careful consideration of about fifty different formulas, which consideration included some actual baking tests.

Before beginning any detailed discussion, it might be well for us to consider what we mean by *experimental or laboratory baking test.* How would you define it? Out of the several definitions the following have been selected as examples.

1. An experimental baking test is a test in which the resultant product shows the combined action of all the ingredients used during the process of preparation and baking.

2. An experimental baking test is that test which will exhibit the value of dough batch ingredients and the purpose for which a dough batch ingredient is used.

3. An experimental baking test is that test which will determine the character and quality of a product that can be produced by using a given formula and method of procedure.

The usual object of a baking test is to determine the quality or value of one dough batch ingredient. In order to accomplish this it is necessary to make the best combination of ingredients and adopt the best method of procedure to bring out the information desired. The results of such tests are interpreted in the light of our past experience.

Right here is the crux of the situation. Each laboratory following its own method secures certain results. These results are comparable with results secured in previous tests. Suddenly or radically to change either formula or method of procedure renders the laboratory operative unable satisfactorily to interpret results or compare them with previous tests. These facts at least make difficult any attempt toward uniformity or standardization. However, it seems to us possible to work out what might be called a standard official method for laboratory baking tests with hard wheat flour which could be used to check against the individual laboratory method and to compare with the results from other laboratories when the standard method is used.

Perhaps one of the first things to decide is the character and quality of bread which should be produced in a laboratory loaf. What character and quality of bread will enable one best to judge the effect or value of a dough batch ingredient? Should the check loaf approach as nearly as possible the character and quality of bread which would be satisfactory in a good commercial bakery or should conditions be exaggerated and a loaf with extreme volume and texture be produced? Additional questions for consideration are: Is there any such thing as proper proportions of ingredients and correct method of procedure? Is there any such thing as the best type or shape of baking pans with proper ratio between pan volume and weight of dough?

It has been well known that there was a lack of uniformity in the formulas and methods of procedure in the various testing laboratories. In order to get an accurate idea of just what differences existed in a large number of well known laboratories, a questionnaire was sent out requesting information in regard to formulas and methods of procedure and dimensions of baking pans used. Nearly every laboratory replied promptly with the desired information.

After accumulating these data some time was required to calculate the various ingredients in percentages based upon the amount of flour used. The results are rather surprising and show the utter lack of anything like uniformity or regard for the proper proportions of ingredients. While considerable latitude may be allowed in proportions of ingredients with a rather wide divergence in the method of procedure it does not seem necessary under any circumstances to have differences as great as these are.

It is not the desire to offer adverse criticism of the formula of any laboratory, as it is not possible to know the reasons for its adoption. However, since we are all working toward the same end, it would seem possible to establish some fundamental process to serve as a guide in working out a standard or official method to be used when making comparative tests with other laboratories. Such a method could be compared with the regular method used in the laboratory and the results interpreted according to experience gained from the regular method.

In studying the various formulas reported, attention is given to the amount of flour, as this ingredient is used as a base upon which the other ingredients are calculated.

Flour

The amount of flour used varied from 300 to 600 grams and includes the following amounts:

315 grams	340 grams	392 grams	600 grams
325 grams	350 grams	400 grams	
336 grams	380 grams	500 grams	

There were 17 formulas calling for 340 grams, 7 for 400 grams, 7 for 500 grams, 4 for 325 grams, 3 for 350 grams, while the other amounts were each used by only one laboratory.

In deciding upon the amount of flour to be used in the standard formula for a single loaf, the following requirements should be made, namely: the amount should be sufficient to give accurate, dependable results, but at the same time, in the interest of economy and on account of possible limited supply, the amount should be as small as accurate work will permit.

Yeast

The per cent of yeast provided for in the different formulas ranges from 1 to 5.88, there being 30 different per cents recorded. Some of these variations were slight, being in the second decimal place, and were probably caused by the endeavor to use a whole number of grams of yeast rather than a fixed per cent. A variation in the amount of flour specified then caused slight variation in per cents of all other ingredients. The amounts most commonly used ranged from 2 to 3 per cent, altho 6 formulas called for from 4 to 4.61 per cent and one formula for 5.88 per cent. On the other hand, we have as low as 1 per cent, and 1.5 per cent.

Increased per cents of yeast will, of course, mature the dough more quickly and thus is a time saver. However, too much yeast is likely to obscure small differences caused by other dough batch ingredients.

Sugar

For a baker's sugar there is probably nothing better than cane sugar for the experimental laboratory loaf. It serves the double purpose of a sweetening material and yeast food. The amount of sugar specified in the different formulas varies from 1.9 to 5.1 per cent with the larger number using from 3 to 4 per cent. Usually the larger per cent of sugar accompanies the larger per cent of yeast, but this is not always the case. The normal ratio between sugar and yeast in a commercial plant is about 3 to 1.

Salt

The per cent of salt shows less variation than all the other ingredients and yet there is probably a better reason for varying the salt than yeast, sugar, or shortening. Salt as a dough batch ingredient gives flavor, acts as a retarder or "governor" of fermentation both diastatic and alcoholic, arrests undesirable ferments, and toughens gluten. It should be borne in mind that salt varies in composition. The amount of salt to use depends upon the character and quality of the gluten and upon mineral content of the water used. When the water is low in mineral content with principally carbonates, more salt is required than when the mineral content of the water is high and consists more largely of sulphates. Salt averaged from 1.35 to 2.12 per cent, most laboratories using 1.5 to 2 per cent. The average commercial plant will use about 1.75 per cent.

Shortening

Shortening is another ingredient which shows considerable variation, 8 laboratories out of 45 not using any while some of the others go as high as 3.33 per cent. The various chemists have given no reason for omitting shortening. Possibly the objection is based on the score of variation in composition of shortening material, but it scarcely seems probable that such variation would be as great when pure lard is used as would be found in any of the other dough batch ingredients. Variations in the composition of the salt or water might easily have a greater effect upon the bread than variations in lard composition.

Shortening performs several functions as a dough batch ingredient. It acts as a sort of lubricant of the dough, aiding in mixing and fermentation. The films of the shortening material prevent evaporation and hence check too rapid staling. There is also a mechanical dispersion of the gluten and there is a certain effect upon the texture of the loaf which gives a combination of tenderness, brittleness, and softness, commonly known as shortness.

Miscellaneous

Only two laboratories used any sort of yeast food in their regular baking tests, two others used malt preparations, the majority evidently preferring to keep the formula free from everything except the basic ingredients, flour, yeast, sugar, salt, water and shortening.

Method of Handling Doughs

The methods of handling doughs must necessarily vary greatly. Part of this is on account of wide divergences of formulas. It is also

much more difficult to give specific written directions for punching or kneading the dough since much depends upon the element of judgment.

The common method is to give first punch when the dough is ready. This is usually ascertained by pressing in the surface lightly with the finger and if the imprint is retained, the dough is then ready. Subsequent punches are then usually given upon fixed divisions of time, such divisions being frequently 60 per cent of the fermentation period for the first punch and later punches at 25 and 15 per cent or 28 and 12 per cent.

Temperature for mixing varied from 80 to 86°F. with like variations in temperature of dough cabinet. The pan proof temperature varied from 80 to 113°F.

The amount of proof was usually stated in time, but this is quite probably the chemist's estimate of the average time required to reach a given height in the pan. The handling of doughs including molding and panning can be demonstrated, but can be described only with difficulty. It is quite probable that such methods have not, in all cases, been properly interpreted from the letters.

In the preparation of ingredients before mixing with the flour, about 5 per cent of the chemists make up a mixture of yeast, sugar, salt, and water in proper proportions and sufficient quantities for the total number of loaves and allow this mixture to stand, drawing off aliquots as needed. Such a procedure is practically equivalent to a pre-treatment of the yeast and if the last aliquot stands from 30 to 60 minutes, it certainly is activated much more than the first aliquot and the time will be markedly affected. If a dozen or more loaves are being baked, following this procedure, it is suggested that a check loaf be put in last as well as first and that the effect on dough time and pan proof time be noted.

Baking Pans

The baking pans are apparently as variable as the formulas. The extremes are the high narrow pans about 6 inches deep and flaring from 2½ to 3½ inches in width and 6½ to 7 inches in length as compared with the low shallow pans 2½ inches deep and flaring from 4 to 5¾ inches in width and from 7 to 8 inches in length.

Attempt was made to calculate the number of cc. of pan volume per gram of dough in the loaf. To do this, it became necessary to assume an arbitrary figure for absorption where none was given. Sixty per cent was assumed. Calculating this ratio gave the range 1.34 to 4.48:1. In only one case is the ratio 4:1 or greater and in only 10 cases is it 3:1 or greater. There is no absolute or fixed rule for the

ratio in commercial baking pans, but the standard size of pan for a 15 to 17 ounce loaf gives a ratio of about 3½ or 4:1. For a larger loaf (24 to 26 ounces) the ratio becomes smaller and ranges from 2¼ to 2¾:1.

In a large number of cases the laboratory baking pans afford much less volume per gram of dough than do regular commercial bakery pans.

From the data that have been secured and presented herewith, it is quite evident that very few laboratories are using anything like the same formula, method of procedure, or type and style of pan. It is possible that still wider variations might be shown if additional questionnaires were sent out. However, it seems that we have enough data at hand to bring the matter before the association and to suggest that a committee be appointed to investigate the possibility and the desirability of working out a standard or official method for baking tests which could be used as a means of checking results between laboratories.

Baking Test Data

No.	Gms. flour	% Yeast	% Sugar	% Salt	% Lard	cc. pan volume per gram of dough	Size of pan		
							Top	Bottom	Depth
1	325	2.46	3.07	1.53	1.84	2.21	8½x4¼	8 x3¾	2¼
2	340	3.82	3.53	1.35	0	3.13	6⅞x3⅝	6¼x2¼	5¾
3	340	2.94	3.53	1.76	2.35	2.45	8 x3⅞	7¼x3¼	2¾
4	340	2.5	3.53	1.54	0	3.35	9¼x4	8½x3	3¾
5	500	4.0	4.5	2	2	3	9¼x4¼	8¼x3	3¾
6	392	2.3	3.06	1.53	2.55	2.8	9¼x5¼	8 1/16x4 3/16	2¾
7	500	2	3.6	2	2.4	2.72	9½x5½	8¼x4¾	3
8a*	400	1.5	4	1.75	1.5	2.04	8⅝x4⅝	7 9/10x3½	2⅝
8b	325	2.46	3.07	1.84	1.84	2.49	8⅝x4⅝	7 9/10x3½	2⅝
9	300	1	3.33	1.66	3.33	4.48	8½x5½	7 x4¾	3½
10†	340	2.64	3.53	1.76	1.76	2.68	9 x3⅞	8¼x3⅛	2⅞
11	340	3.53	2.94	1.76	1.47	2.5	8½x4½	7½x3½	2¾
12a	500	1.75	2.5	1.75	2	....	.....	.....	...
12b	500	3	2	1.75	1.7	....	.....	.....	...
13	354	2	2.5	1.5	0	2.44	9 1/16x4¼	7⅞x3¼	2¾
14a	340	2.35	2.94	1.47	0	2.8	8 1/16x4½	7 1/16x3½	3¼
14b	340	3.53	2.94	1.47	0	2.79	8 1/16x4½	7 1/16x3½	3¼
15	350	2.29–3.42	2.85	1.42	2.84	3.23	7 x3¾	6 x2¾	5½
16	500	2.4	2	1.4	1	2.73	9½x5	8½x4	3
17	336	4.16	4.16	1.93	2.8	3.41	7 x3⅝	6¾x2½	6
18a	340	4.41	3.53	1.47	2.94	2.9	6¾x3⅝	5⅛x2⅞	5 5/16
18b	315	1.9	1.9	1.42	1.9	2.05	7 11/16x3 11/16	7 1/16x3⅛	2½
19	340	3.75	3.75	1.65	3.125	3.49	.....	.....	...
20	340	3.53	3.53	1.76	2.35	2.23	8½x4½	8½x3	2½
21	450	3	3	1.5	1.5	2.49	8¾x4	8 x3	3
22	400	2.62	3.51	1.5	2	2.8	8¾x5	8⅛x4⅛	3
23	400	2	3	1.5	1.5	2.33	8½x5¾	7 x4	2½
24	340	2.94	4.41	1.47	0	3.2	6½x3¾	5⅛x2¾	5½
25	500	3	2	1.4	0	1.34	8¾x4 1/16	8 x2 1/16	2½
26	325	4.61	4.61	2.12	3.07	2.5	9¼x3¾	8½x3	2⅞
27	500	3	3	1.8	1	2.66	9⅛x4⅞	8⅞x4⅝	2¾
28	340	2.35	3.01	1.76	2.94	2.02	8½x4¼	8⅛x3¾	2⅜

No.	Gms. flour	% Yeast	% Sugar	% Salt	% Lard	cc. pan volume per gram of dough	Size of pan		
							Top	Bottom	Depth
29	340	3.53	3.53	1.76	2.64	2.51	8½x4½	7⅝x3⅝	2¾
30a	380	2.63	3.94	2.1	2.63	2.86	9¼x4	8¼x3	3¾
30b	380	3.16	3.94	1.84	2.63	2.89	9¼x4	8¼x3	3¾*
31	350	3	3	1.5	1.57	2.23	8¾x4	8 x3	3
32	350	4.26	4.26	1.42	1.42	2.32	8¼x4¼	8¼x3⅞	2½
33	340	5.88	4.41	1.47	1.47	3.27	6⅝x3½	5⅞x2¾	5⅝
34	600	3.33	3.33	1.66	1.66	2.42	9½x5½	8¼x4¼	2¾
35	340	3.53	4.11	2.05	2.05	3.41	7 x3½	6½x2½	6
36	350	3	3	1.5	1.5	2.07	9¼x4⅛	8⅝x3¼	2¼
37	392	4.59	5.1	1.78	2.55	2.60	9½x5	7½x3	3⅛
38	340	3.53	3.53	1.47	2.35	2.97	5¾x3¼	5 x2½	5½
39	400	3	3.75	1.75	2.5	2.80	9½x4	8½x2⁹⁄₁₀	3¾
40	400	4	4	2	2	3.03	9¼x4	8¼x3	3¾
41	400	3	3	1.5	1.75	2.42	6 x3½	5 x2¼	6¼
42	340	2.94	4.41	1.47	1.47	2.55	8½x4½	7⅝x3⅝	2¾
43‡	400	2.5	3.12	1.5	0	....	.....	.....	...
44§	400	2.5	1.25	1.75	2	2.04	8⁷⁄₁₆x7⁷⁄₁₆	4¾x3⁷⁄₁₆	2⅛
45	340	2.94	4.41	1.47	0	3.22	6½x3⅞	5½x3	5½

*Arkady 0.62% used.
†Diamalt 0.88% used.
‡Arkady 1% used.
§Milk powder 2.87%, malt 1.25% used.

No.	Type mixer	Mixing: Time, min.	Mixing: Temp., degrees	Mixing: Dough temp.	Approx. time of punches, minutes: 1	2	3	4	Minutes bench proof	Pan proof: Time, min.	Pan proof: Temp., degrees	Baking: Time, min.	Baking: Temp., degrees	Misc.
1	Bachman	2½	80F.	.....	×	×/2	×/4		10	.....	90	.....	475–500	
2		...	..		20 min. after kneading	65	..	..	.....	120	88	35	.....	§
3		...	..	.....	90	45	..	..	.....	75	85	35	180–200C.	
4		...	..		60% time	28% time	12% time	...	.....	.....	36C.	30	220C.	
5	Hobart	5	80	.....	90	30	30	..	10	60	.....	.....	.....	
6	Hobart	4	80	80F.	100	50	30	..	.....	.....	95	.....	.....	
7	Hobart	4	..	84	90	45	30	..	.....	.....	.....	.....	.....	
8a		...	..	.....	120	40	20	..	20	50–55 90	95	Commercial pans High pans		
8b		...	..	.....	120	50	20	..	20	90–95	95	.....	.....	
9		3	30C.	.....	150–180	..	..	..	12	50–70	45C.	30	210C.	
10	Hobart	4	78–79	.....	80–100	40	30	..	15	50–60	95	22	460	
11	Hobart	4	82	.....	110	40	..	..	15	60	86	30	425	
12a	No information	...	..	80	...	..	..	..						
12b	No information	...	..											
13	Bachman	5	..	80	Rise until finger will leave imprint. Repeat this				15	Until little spring left	90 ..	.....	450	
14a	Bachman	Until	85	.....	80	20	..	..	15	45–55	88	.....	420	
14b	Same	uniform	..		Varies									
15		†	..											
16		...	..	80	150	60	..	..	45	40	95	.....	.....	Scaled
17		1½	..	82	60	30	..	..	30	85–90	82	.....	.....	
18a	Bachman	5	..		10 folds	6 folds			.....	.....	85	60	180C.	
18b	Same‡	...	..											
19	Hobart	...	..	80	35–50	20	..	..	15	60	95	.....	.....	
20	Hand mixed	...	..	82	90	30	15	..	.....	65	82	.....	.....	
21	Hobart	3 or	..											
	Werner	4	..	86	60%	20%	10%	..		Exp. test	86	30	200C.	
22	Hobart	3	80–81	80	60%	25%	15%	..	10–12	.....	90	.....	400	
23	Hobart	4	80	82–84	120	75	..	..	30	55	88–90	40	400	

No.	Type mixer	Mixing: Time, min.	Mixing: Temp., degrees	Mixing: Dough temp.	Approx. time of punches, minutes: 1	2	3	4	Minutes bench proof	Pan proof: Time, min.	Pan proof: Temp., degrees	Baking: Time, min.	Baking: Temp., degrees	Misc.
24	Hobart or Werner	...	90F.	.....	80	20	..	..	.....	75–120	90	35	400–425	Sponge
25	New Era	10	78F.		3 hours for 3 punches				15	60	82	.....	.....	
26	Fleischmann	3	30C.	.....	120	40	20	..	.....	60	33–34C.	20–30	425–450	
27	Hobart	4	80F.		60% fer	25% fer.	15% fer.		.....	.....	90F.	30	500–510	
28	Hobart	2½	81	.....	90	60	30	..	20	60	95	35	460	
29	Hand-mixed	...	80	.....	70	30	..	..	20	55–60	98–100	.....	.....	
					(1)*	(2)*								
30a	W-P	6	80	80-82	90–100	30	15	..	10	40–50	93	30	450	
30b	W-P	10	80S.	80-82	110-120	55-60			15	40–50	93	30	450	
			W.	80-82	95-105	45-50			15	40–50	93	30	450	
31		...	86		200 min. until moulded				.....	90	93	.....	.....	
32	Hand-mixed	...	82	82–84	90	45	..	..	20	.....	90	.....	430	
33	Hobart	4	85	.....	45	35	..	..	10	55–75	90	45	400	
34		5	82	82	120	60	45	15	15	.....	90	.....	450	
35	Hand-mixed	...	..	82–83	55	30	..	..	20	85	90	.....	200–250C.	
36	Read	...	80		65% time	25% time			10% time	50–65	90	20–25	300	
37		...	..	.....	90	30	10	..	10	.....	.....	.....	.....	
38	Hand-mixed	...	80	.....	120	40	20	..	20	60	80	.....	359–400	
39	Universal	130–150 turns	80		Until max. exp. is almost reached.		45	..	..	.....	50	88	35–45	200C.
40	Hobart	6	80–83	.....	30	45	30	..	15	60	.....	30	420–430	
41	Hobart	2	82–83	Time between punches regulated by volume of dough.						Proof regulated by volume.				
42		...	90		Max. exp. ⅓ time for 1st				.....	.....	.....	40	200C.	
43		Until smooth	80	.....	120	60	..	..	20	50	93–95	.....	.....	
44	Hobart	...	80	.....	115	60	30	..	15	60	.....	30	.....	

*(1) First punch, (2) second punch.

†Three hours fermentation time with three cutovers.

‡Except that bread was baked in shorter time and hotter oven.

§Sponge fermented at 88° after 1 hour 40 minutes, add sugar and knead.

COUNTING YEAST CELLS IN DOUGH

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There is considerable lack of agreement in baking literature on the growth of yeast in dough. This is partly due to the lack of satisfactory methods for counting yeast cells in dough and also to the omission by the investigators of necessary details regarding conditions of fermentation. An examination of the literature available to us failed to show that any considerable work had been done in the development of methods for counting yeast cells in dough, with the exception of the work of Neumann and Knischewsky (1).

There are, however, methods still unpublished which are used in commercial laboratories. These will not be discussed here.

Purpose of the Method

This lack of information in the literature led the writer to attempt to work out a more satisfactory method for counting yeast cells in dough, which would be useful in research and technical investigations, particularly in connection with problems of yeast nutrition, commercial "yeast foods," and bread improvers. Many studies of yeast nutrition published in the literature of bread making have been confined to nutrient solutions and other culture media and apparently little work has been done in connection with actual studies of yeast in such a complex habitat as dough.

The method of Neumann and Knischewsky (1) is as follows: "Different amounts of yeast were added to a dough consisting of 74 grams of flour and 45 of water. A piece of dough weighing 15 grams was removed from the original dough and placed in 150 cc. of water. The gluten separated by washing 25 cc. of this solution was mixed with 25 cc. of iodine solution (150 cc. water + 1 gram K I + 0.05 gram I) and diluted to 400 cc. with distilled water. One drop of this solution was removed with a pipette and placed on the ruled area of a yeast-counting apparatus or ordinary blood-counting chamber. The ruled area on the slide contains 20×20 squares. The yeast cells were counted in a row of 20 squares so each figure in the table below gives the number of yeast cells in 20 squares. Three different slides were made from the same sample of dough and water.

To obtain a count for 5 squares, the 400 squares are divided by 80.

Dough plus 4 grams yeast.

Preparation 1					Preparation 2					Preparation 3				
27	17	34	33		29	20	30	15		21	24	21	19	
27	27	23	38		20	25	29	16		21	32	25	27	
24	20	15	27		25	26	28	16		26	20	22	16	
25	30	33	19		21	32	18	21		24	37	24	29	
25	27	22	26		20	35	28	24		24	24	23	21	
128	121	127	143	Total 519	115	138	133	92	Total 478	116	137	115	112	Total 480

519 divided by 80 = 6.48 cells in 5 squares
478 divided by 80 = 5.97 cells in 5 squares
480 divided by 80 = 6.00 cells in 5 squares

18.45 divided by 3 = 6.15 cells in 5 squares.

The author attempted to use the above method, but found that it was unsatisfactory because of the difficulty experienced in washing all the yeast out of the gluten, as is shown in the following experiments. The bread formula was that used by O. W. Hall, in charge of the technical and service department of the Institute.

Flour, 325 grams
Water, 195 cc. based upon 60 per cent absorption
Yeast, 8 grams
Sugar, 10 grams
Salt, 5 grams
Lard, 6 grams

Total, 549 grams (weight of dough when mixed).

According to this formula there are present 0.0145 grams of yeast per gram of dough. Fourteen and one-half milligram portions of yeast were weighed in a covered glass weighing dish. Bits of yeast were taken from various parts of pound cakes of yeast. The weighed yeast was transferred to a 600 cc. beaker. Two drops of water from a 100 cc. portion were added to the beaker and the yeast and water thoroly mixed with a glass rod. The remaining portion of water was added to the beaker and the whole thoroly stirred with the glass rod. A drop of this yeast suspension was placed upon the ruled area of a blood-counting cell, observing the following precautions: The proper amount of suspension was placed upon the center of the cell so that it was entirely covered. An excess should not be used in order that the liquid will not spread upon the sides of the counting chamber. The solution was thoroly stirred before taking the sample. Air bubbles were avoided in mounting the preparation.

The cover glass was tightly fitted to the counting chamber. The calculation of the number of yeast cells in 0.0145 gram of yeast was also determined from 0.1 gram and 1 gram portions of yeast weighed in a glass weighing dish with cover. Four different slides were made

from the same yeast preparation. The yeast cells were counted in a row of 20 squares, and 6 rows were counted in each preparation starting with the top row and counting every other row. It was determined that the average number of yeast cells in .0145 gram of yeast is 114,000,000. A typical determination is given in the table below.

4	3	13	5	27	
4	7	8	11	30	
7	10	4	1	53	
4	7	13	5	27	
2	1	13	2	137	
6	2	2	3		137 ÷ 24 = 5.7 cells in 20 squares
27	30	53	27		

5.7 × 20 = 114, number of cells in 20 rows.
114 × 10 × 1000 = 1,140,000, number of cells in 1 cc. of liquid.
100 cc. in preparation.
1,140,000 × 100 = 114,000,000 cells in .0145 gr. of yeast.

The writer used the Neumann and Knischewsky method on pieces of dough taken from one-pound doughs prepared in the service laboratory, and found that the counts fell short from 10 to 30 million yeast cells, based on the number calculated per one gram of dough as above. In the table below is given a series of counts on doughs made up with various percentages of yeast. The calculated number of yeast cells in one gram of dough is given in the second column. Each figure represents the average found in three preparations.

Dough No.	Yeast, per cent	Theoretical No. that should be found 2.85	Found by Neumann method, 2.41	Enzyme method, 273
1	2.46	114,000,000	86,400,000	109,200,000
2	2.00	92,600,000	70,000,000	94,080,000
3	2.46	114,000,000	98,000,000	110,400,000
4	2.46	114,000,000	82,800,000	116,000,000
5	3.00	139,000,000	122,000,000	139,600,000
6	2.46	114,000,000	91,400,000	108,800,000
7	2.46	114,000,000	88,400,000	111,000,000
8	1.75	81,000,000	68,000,000	82,600,000
9	2.00	92,600,000	72,800,000	92,000,000
10	2.46	114,000,000	88,200,000	112,400,000
11	2.46	114,000,000	96,800,000	117,200,000
12	2.46	114,000,000	94,600,000	112,200,000

The writer has examined washed gluten microscopically from samples obtained from the service laboratory and observed many yeast cells embedded in the gluten. This observation was confirmed on the washed glutens obtained by the Neumann and Knischewsky method. The writer has been unable to remove all the yeast cells from the gluten as described in their method.

Method 2

The Neumann and Knischewsky method was modified by placing a known weight of dough in 100 cc. of distilled water contained in a 500 cc. brass Erlenmeyer flask. Several pieces of sharp steel were placed in the flask for the purpose of breaking down the dough. The flask was shaken vigorously for three minutes. The contents were then poured into a 250 cc. beaker and a drop of the suspension was examined on a slide and also in a blood-counting cell. Small fragments of gluten concealed some of the yeast cells and covered completely large spaces of the ruled area of the counting cell.

As the objection to the Neumann and Knischewsky method appeared to be due largely to the observation that the yeast cells were obscured by the gluten, which also interfered with the counting area, it appeared evident that such conditions could be obviated by treatment of the gluten in such a way that the yeast cells would not be affected. The use of protein-splitting enzymes, as pepsin, trypsin, and papain, was thus naturally suggested.

One-gram portions of dough were weighed out and placed in 600 cc. beakers. Two hundred cc. of distilled water was added to the beaker at the temperatures stated below for the different enzymes, and the preparation was held at that temperature by means of a water bath. The time consumed in a complete breaking down of the gluten was as follows: pepsin 17 minutes at 60°C., trypsin 20 minutes at 50°C., papain 15 minutes at 65°C. In the above series of digestions, the pH was not determined. In the following experiments the reaction of the medium was adjusted to a definite pH for each enzyme preparation.

In the digestion with pepsin the medium was adjusted to a pH of 2.6 with $\frac{N}{1}$ HCl using thymol blue as the indicator. At this pH a complete breaking down of the gluten took place in 15 minutes.

When the papain was used the medium was adjusted to a pH of 5.2 with $\frac{N}{1}$ lactic acid using brom cresol purple as the indicator. A complete breaking down of the gluten was secured in 12 minutes. This particular papain contained a gritty substance which made it objectionable for use in the blood-counting cell.

Digestion with trypsin was carried out at a pH of 8, using Na_2CO_3 for adjusting the reaction of the medium, and thymol blue as the indicator. Seventeen minutes were required to break down the gluten completely.

In order to determine whether the action of the enzymes dissolved or distintegrated the yeast cells, the following experiments were made.

A suspension of yeast cells and water was prepared using 20 mg. of yeast in 100 cc. of distilled water. The number of yeast cells per cc. of liquid was determined with the blood-counting cell. Each enzyme preparation was tested as follows: As soon as the count had been made a half-gram portion was added and held at 55°C. for 15 minutes. The number of yeast cells per cc. was then calculated. It was found that this treatment did not appreciably affect the cells or reduce the number per cc.

Following this experiment a yeast suspension was made with approximately 25 yeast cells per field, using the pepsin, trypsin, and papain respectively. Certain yeast cells in the preparation were observed for 20 minutes in order to see if any cells disappeared from view or if disintegration took place. There appeared to be no appreciable effect on the living cells and during the time of the experiment only a slight effect was observed on some of the dead cells. On an average we have observed 1 per cent of dead cells in commercial compressed yeast. The action of the enzymes upon the dead cells was not sufficeint to destroy their identity.

In the development of the method of counting using enzyme preparations, it was necessary to find a toxic agent for the suppression of cell multiplication so that any increase in cells during the count would not occur. Mercuric chloride is obviously inapplicable because it combines with the proteins. The toxic agent selected should not cause cell disintegration and should not reduce the stain selected, but on the other hand tend to fix the preparation, producing intensity of color. The stain selected should stain as few particles as possible other than the yeast cell.

The following compounds were investigated: Toluol, formalin, lysol, sterilac (a commercial chloramine disinfectant), and phenol. It was found that phenol was most satisfactory.

Considerable work was done on the selection of a satisfactory stain. Iodine as used in the Neumann and Knischewsky method proved unsatisfactory because it stained both starch granules and yeast cells.

Methylene blue was found to be a satisfactory stain in connection with phenol as a killing agent, as only a small amount is necessary to stain the cells killed by phenol, while the starch granules are not appreciably affected and the yeast cells are stained a light or dark blue.

The proper quantity of phenol and methylene blue was determined by making up suspensions of yeast cells and wheat starch, and finally by actual trial with dough preparations. In a total of 5 cc. of the dough preparation the amounts used were as follows: 0.05 cc. of phenol and 0.15 cc. of methylene blue.

Method for Counting Yeast Cells in Dough

Sampling.—In order to obtain representative samples for examination, 20-gram pieces of dough are collected from different places from the dough batch and placed in a large moisture dish with cover. A one-gram sample of dough is weighed in a glass weighing dish on the analytical balance. This sample is obtained by taking bits of dough from various pieces in the large dish. It was determined by actual count that there is not a great variation in the number of yeast cells per gram in different places in the same dough; especially in a well mixed dough. This was also observed by Neumann and Knischewsky.

Preparation of the sample.—The sample is placed in a 600 cc. beaker with 200 cc. of distilled water (acidified with 0.1 cc. $\frac{N}{1}$ HCl) at a temperature of 65°C. Soluble pepsin, 0.5 gram, is added and the preparation held at a temperature of 65°C. for 15 minutes. This may be done in a water bath. At intervals this digestion is aided by breaking up the small pieces of dough with a glass rod. Digestion is complete in 15 minutes. The digestate is then stirred thoroly and 10 cc. withdrawn by means of a 10 cc. pipette graduated to tenths. Of the amount withdrawn, 4.8 cc. is then transferred to a 20 cc. beaker and 0.05 cc. of melted phenol is added, holding the beaker 1 minute at 65°C. with stirring. The phenol is allowed to act for an additional two minutes. Immediately 0.15 cc. of Loeffler's methylene blue is added and allowed to act for one minute. The sample is then thoroly stirred and one drop (0.01 cc.) transferred to the ruled area of a blood-counting cell.

Counting and Calculation of Results

The yeast cells in a row of 20 squares were counted and recorded. Every other row was counted until the count in 5 rows had been recorded. Two more slides were counted from the same preparation. The total number of yeast cells divided by 15 gives the average number in a row of 20 squares. A typical calculation is given in the table below.

5	1	2	15
4	3	3	12
5	2	2	17
2	3	7	44 ÷ 15 = 2.96 average number of cells in 20 squares.
1	3	1	
—	—	—	
17	12	15	

2.96 × 20 = 59.2, number of cells in 400 squares.
59.2 × 10 = 592, number of cells in 1 cu. mm. of liquid.
592 × 1000 = 592,000, number of cells in 1 cc. of liquid.
1 gr. of dough in 200 cc.
592,000 × 200 = 118,400,000 yeast cells in 1 gram of dough.

Summary and Conclusions

The above method has proved satisfactory for counting the number and studying the increase of yeast cells in dough.

1. We were unable to secure consistent and accurate results in the counts when we attempted to use methods which removed the yeast from the gluten by washing with water. The number of yeast cells found by such methods did not check with the calculated number based upon counts made on known weights of yeast.

2. The use of pepsin makes it possible to break down the proteins so that the yeast cells are readily counted without interference from large particles of gluten obscuring the field.

3. Pepsin does not affect the living yeast cells under the conditions stated nor disintegrate the cells killed by phenol to such an extent that their identity is lost.

4. Dead cells are easily stained and starch granules remain unstained, so that the identity of these elements in the field can be easily observed.

Reference

1. Neumann, M. P., and Knischewsky, Olga. Zeitschrift für das Gesamte Getreidewesen No. 5, p. 113 (1909).

EFFECT OF WATER CONTAINING FREE CHLORINE IN BREAD MAKING

By C. B. Morison

American Institute of Baking, Chicago, Ill.

(Read at the Convention, June 11, 1924)

The chlorination of public water supplies as a protective measure against typhoid and other water-borne diseases is now regarded as standard practice by health authorities and sanitarians. The reduction in the death rate from typhoid fever following chlorination has become a fact of common knowledge. The city of Chicago, during the first nine months after the complete chlorination of the water supply in 1916, reduced its death rate from typhoid fever by 72.44 per cent (1). It is therefore not surprising that chlorination has been generally recommended for the disinfection of raw waters.

The literature of chlorination from the sanitarian's viewpoint is rather extensive. Bartow and Legendre (2) have discussed the history and applications of liquid chlorine in an interesting monograph. There is, however, but scant reference in the literature to the effects of chlorinated water in fermentation industries such as baking and brewing. The writer has been unable to find any specific information on

this subject in the literature of baking thus far examined, altho in conversation and correspondence with practical bakers the general opinion seems to be that the chlorination of water is detrimental to fermentation and quality bread. Many inquiries regarding the effects of chlorinated water in bread making have been received at the Institute, with complaints that when the ordinary dosage of chlorine is increased over the normal amount by the health authorities, fermentation troubles occur, with the production of bread of undesirable characteristics, particularly of flavor and taste.

In view of such inquiries and complaints, and the apparent lack of information on the subject in the literature, an investigation was begun at the Institute on the effects of known amounts of free chlorine in water when applied under the conditions of baking tests.

Experimental

1. **Solution of chlorine.**—A solution of chlorine water was prepared by passing the washed gas generated from C. P. concentrated hydrochloric acid and potassium permanganate into distilled water. The amount of free chlorine in the solution was determined volumetrically by the usual method with potassium iodide and standard sodium thiosulfate solution. The required amount of the chlorine solution was then added to the proper volume of Chicago tap water for the required concentration of free chlorine as expressed in parts per million of water, making a correction for the amount of free chlorine in the Chicago tap water as determined colorimetrically by the *o* tolidine method (3).

2. **Formula and baking tests.**—The formula was in general, except as indicated, the usual one employed in the American Institute School of Baking, as follows:

	Grams
Flour	325
Sugar	10.0
Salt	5.5
Yeast	11.0
Water, according to absorption	195.0
Shortening	6.5

The flour was a Northwestern spring patent of a well known commercial brand.

	Per cent
Moisture	13.24
Ash	0.43
Protein	12.07

The doughs were mixed by hand and fermented, proofed, and baked, in the fermentation cabinet, proofing cabinet, and electric ovens of the School of Baking. The bread was scored by several individuals.

3. **Baking tests with chlorinated water, Series 1.**—Three doughs were made with Chicago tap water containing 0.3 parts free chlorine per million as determined by the *o* tolidine method.

Average temperature of dough, 81°F.
Average total fermentation period, 140 minutes.
Average score of bread, 90.5.

Series 2.—Three doughs were made with Chicago tap water containing 1.0 part free chlorine per million. The free chlorine content of the water was increased from 0.3 to 1.0 part by the addition of the required amount of chlorine water.

Average temperature of dough, 81°F.
Average total fermentation period, 140 minutes.
Average score of bread, 90.

Series 3.—Three doughs were made with Chicago tap water containing 5.0 parts of free chlorine per million. The free chlorine content of the water was increased from 0.3 parts by the addition of the required amount of chlorine water.

Average temperature of dough, 82°F.
Average total fermentation period, 138 minutes.
Average score of bread, 89.5.

Series 4, A.—Three doughs were made as follows: (1) Chicago tap water 0.25 parts free chlorine per million; (2) Chicago tap water 10 parts free chlorine per million; (3) Chicago tap water 10 parts free chlorine per million. In (2) and (3) the free chlorine content was increased from 0.25 parts by the addition of the required amount of chlorine water.

Test 1.—0.25 parts chlorine per million.
Temperature of dough, 80°F.
Total fermentation period, 133 minutes.
Score of bread, 90.5.

Test 2.—10 parts chlorine per million.
Temperature of dough, 80°F.
Total fermentation period, 133 minutes.
Score of bread, 88.

Test 3.—10 parts chlorine per million.
Temperature of dough, 80°F.
Total fermentation period, 133 minutes.
Score of bread, 88.

This series of baking tests was made by Student A, who reported that the doughs appeared to "tighten up" with water containing 10 parts free chlorine, that they had a bleached appearance, but that there was no perceptible bleaching of the crumb noticed in the bread.

The bread was scored by several individuals, independently, who

gave the score of 13.5 for flavor and 18 for taste to the three loaves of bread.

This series of tests was repeated independently by Students B and C, whose results agreed well with those of A. The bread scored as follows:

	Student B	Student C
	0.30 parts chlorine per million	0.30 parts chlorine per million
Test 1	88	89.5
	10.0 parts chlorine per million	10.0 parts chlorine per million
Test 2	91	90
Test 3	91	89.5

No appreciable effect on the flavor and taste was noted, both students scoring these 14 and 18, respectively, for each of the three loaves. The volume was somewhat higher in the bread made with water containing 10 parts of free chlorine per million. Student B reported a volume of 1850 cc. against 1800 cc. with the low chlorine content, and Student C 1825 cc. against 1800 cc. Slight variation was noted in the total time of fermentation of the three doughs, 167 minutes for 0.30 parts chlorine per million and 161 minutes for the dough containing 10 parts chlorine per million.

A fifth series of baking tests was run with Chicago tap water containing 20, 40, 80, and 100 parts free chlorine per million respectively. These amounts are so abnormal in relation to the amount of residual free chlorine in Chicago water which ranges from 0 to 0.4 parts chlorine per million that we refer to them merely as a matter of interest.

The quality of the bread made with water containing these abnormal amounts of free chlorine was surprisingly good in respect to external characteristics, but in each test the flavor and taste were characterized by a rather strong pungent aromatic quality similar to that associated with certain chlorinated products. The period of fermentation appeared to be shortened by these excessive amounts of free chlorine, but we have not sufficient data at this time for a satisfactory discussion of such effects.

In addition to the above laboratory baking tests, doughs were run on a larger scale in the Institute bakery under our shop conditions. The following experiment is reported in this connection.

There was but little difference in the quality of the bread made from the Chicago tap water containing 0.35 parts of free chlorine per million, and that made from the same water the free chlorine content of which had been increased to 5.0 parts per million. In this experiment the score of the bread was slightly higher for dough 3, containing the high amount of chlorine.

	Dough 1 Formula Water contained 0.35 parts chlorine per million	Dough 2 Formula Water contained 0.35 parts chlorine per million	Dough 3 Formula Water contained 5 parts chlorine per million
Flour	100	100	100
Water	58	58	58
Cerelose	3	3	3
Crisco	1.50	1.50	1.50
Salt	1.75	1.75	1.75
Diamalt	1.00	1.00	1.00
Arkady	0.25	0.25	0.25
Time of mixing	12 mins.	12 mins.	12 mins.
Temperature	82°F.	82°F.	82°F.
Total time of fermentation	135 mins.	120 mins.	115 mins.
Score of bread	89.0	89.5	92.0

4. **Gas production.**—It seemed to be of interest to determine the effect of the presence of free chlorine in the 10 per cent sugar solution used in the Hayduck method for the determination of the fermentation power of yeast. A 10 per cent solution of cane sugar in distilled water was prepared, and in one portion of it an amount of chlorine water was added so that the resulting solution contained 5 parts chlorine per million. Fermentation tests were made with 400 cc. of the 10 per cent sugar solution at 30°C., and at the same time with the same amount of the sugar solution containing 5 parts of free chlorine per million. Ten grams of compressed bakers' yeast (Fleischmann's) taken from the interior portion of the cake, was used in each test. In a series of six Hayduck tests the average amount of gas collected in 72 minutes was 242 cc. for the 10 per cent cane sugar solution free from chlorine, and 244 cc. for the sugar solution containing 5 parts chlorine per million.

The sugar solutions containing chlorine had the characteristic odor after fermentation associated with chlorinated products, but no evidence of free chlorine could be detected by either the starch potassium iodide test or by *o* tolidine. Tests for chlorine were also negative immediately after the addition of the chlorine water to the sugar solution before connecting up with the gasometer.

A series of fermentation tests was also made after the method of Meissl, using 1 gram of yeast, 4 grams of cane sugar, and 0.5 gram each of potassium phosphate and ammonium phosphate in (1) 50 cc. of distilled water, (2) Chicago tap water containing 0.3 parts chlorine per million, and (3) Chicago tap water containing 5 parts chlorine per million. The solutions in the Meissl flasks with sulfuric acid traps were weighed and incubated for 24 hours at 30°C. At the end of this period, the flasks were again weighed and the losses were as follows:

1. Distilled water.....Loss, 1.51 grams
2. Chicago tap water, 0.30 parts chlorine per million..Loss, 1.53 grams
3. Chicago tap water, 5.0 parts chlorine per million...Loss, 1.54 grams

Conclusions

The results of the baking tests indicate that the presence of free chlorine in water to the extent of 5 to 10 parts per million does not appear to have a deleterious influence on bread quality. Such amounts would be from twelve to twenty-five times the maximum amount of residual chlorine that we have found in the Chicago water supply at our laboratories.

The presence of 5 parts per million of chlorine in the water used for the determination of the fermentation power of fresh yeast by the Hayduck and the Meissl methods, did not appear to decrease the rate or total volume of gas evolved in comparison with tests made at the same time and under the same conditions in which no free chlorine was present.

The writer wishes to express his appreciation of the assistance of Messrs. Walmsley, Jackson, Thompson, and Schneider, of the School of Baking, in conducting the baking tests.

References

1. Chicago Dept. Health Bul. 42 (1923).
2. Bartow, E., and Legendre, R. La Chloration. pp. 1-32. Paris (1918).
3. Am. Pub. Health Assn. Standard methods for the examination of water and sewage. Laboratory Section. pp. 44-45 (1921).

CONSTITUTION OF THE AMERICAN ASSOCIATION OF CEREAL CHEMISTS

Article I. Name

The name of this organization shall be THE AMERICAN ASSOCIATION OF CEREAL CHEMISTS.

Article II. Purposes of the Association

The purposes of this association are (1) the encouragement and advancement of scientific and technical research in cereals and their products, particularly in milling and baking, but including also other industries in which cereals and their products are utilized, (2) the study of analytical methods used in cereal chemistry and the development and adoption of uniform (or standard) methods of examination and analysis, (3) the promotion of the spirit of scientific co-operation among all workers in the field of cereal knowledge, (4) the maintenance of high professional standards in the association as conditions of membership and (5) to encourage a more general recognition of the chemist and biologist as essential factors in the development of the cereal industries. In accordance with these purposes this associa-

tion shall conduct a journal in which contributions to the scientific knowledge of cereals, their products and technical application shall be published for the encouragement and advancement of the science. It shall hold annual or other meetings for the discussion of cereal knowledge and the promotion of research and technical co-operation among its members.

Article III. Membership

Section 1. The membership of this association shall be divided into four classes, active, associate, honorary and sustaining.

Sec. 2. The active membership shall be restricted to those persons having had at least two years of chemical training in some accredited school or the equivalent experience.

Sec. 3. All applications for membership must be passed upon by a body known as the executive committee, their decision to be final.

Sec. 4. Those persons having one year's experience in laboratory shall be admitted as associate members at the same fee as active members. Their qualifications shall be determined by the executive committee. Such members are not to have any active part in the business meetings of the association.

Associate members can become active members after five years of continuous membership upon unanimous recommendation of the executive committee.

Sec. 5. Honorary members may be elected by a three-fourths majority vote of the members present at a regular meeting, the name of the candidate to be entered by an active member of the association.

Sec. 6. Application for membership must be made in writing and shall be proposed by at least one active member of the association.

Article IV. Officers

Section 1. The officers of this association shall be president, vice-president, secretary-treasurer, managing editor, and chief of the editorial staff.

Sec. 2. Election of officers shall be by ballot at general meetings. There must be at least three nominations of active members for each office to make the election valid.

(a) In order to be declared elected the nominee must secure a majority of all the votes cast.

Sec. 3. Duties of officers.

(a) The president shall preside at all meetings and be the official head of the association.

(b) The vice-president shall preside at all meetings in the absence of the president and assist him in the duties of the office. He shall also act as chairman of the executive committee.

(c) The secretary-treasurer shall keep a record of the minutes of the meetings, send out notices to the members and handle all correspondence of the association. He shall collect all fees and money due the association and pay all bills by check, such bills and checks to be countersigned by the chairman of the executive committee.

(d) The president and the chairman of the executive committee shall jointly select three active members of the association to act as an executive committee.

(e) It shall be the duty of this committee to investigate the qualifications of applicants for membership. This committee shall report to the association in general session. The committee shall co-operate with the president in carrying on the business of the association between meetings. It shall be the privilege of the president to vote in the meetings of this committee.

(f) The president shall appoint three active members to act as an auditing committee. This committee shall report at all regular sessions. It shall audit the books of the association at any time by request of the president.

(g) The managing editor shall publish the journal.

(h) The president and the chief of the editorial staff shall appoint a suitable number of active members to act as an editorial staff.

(i) The editorial staff shall prepare the material for the official organ of the association and submit same to the managing editor for publication.

Article V. Fees

Section 1. The application fee shall be ten dollars which shall include first year's dues. The fee must accompany the application, the fee to be returned in case the application is rejected or applicant fails of election.

Sec. 2. The dues of this association shall be five dollars ($5) per annum, from January 1 to December 31. This shall include subscriptions to all publications of the association.

Sec. 3. Honorary members shall be exempt from dues and fees.

Sec. 4. Assessments, not to exceed one year's dues, may be levied when the current expense of the association makes this necessary. The treasurer with the consent of the executive committee may levy said assessment.

Sec. 5. Failure on the part of any member to pay his dues or assessments within one year after due shall be regarded as resignation.

Sec. 6. All annual dues must be paid in advance, the membership card constituting a receipt for same.

Article VI. Elections and Meetings

Section 1. Meetings shall be held annually at such time and place as may be determined by the executive committee.

Sec. 2. In all general meetings an attendance of at least one-third of the active members of the association registered at the meetings shall constitute a quorum.

Sec. 3. The officers shall be elected to serve for a term of one year or until successors are elected.

Sec. 4. Honorary and associate members shall have the privilege of attending all general meetings and the privilege of the floor, but shall have no vote.

Article VII. Amendments

Section 1. Amendments to this constitution may be made at any general meeting; a two-thirds majority vote of the members present shall be necessary to carry.

Amendment I

Article V, Section 2, be amended as follows: The annual dues of this association shall be five dollars per year, payable to the secretary-treasurer, of which three dollars shall constitute a subscription to the journal of the association, CEREAL CHEMISTRY, and shall be set aside as such. Said dues shall be payable strictly in advance and if not paid by the time of the March issue of the journal said delinquent member shall be dropped from membership.

Amendment II

Article IV, Section 2 (e), be amended as follows: All applications for membership in this association, where the qualifications of said applicant fully meet the requirements as set forth in the constitution, shall be approved by the secretary-treasurer of this association. Only applications where qualifications are doubtful shall be submitted to the executive committee.

BOOK REVIEWS

Practical Milling, by D. W. Dedrick, Pennsylvania State College, State College, Pa. Published by The National Miller, Chicago, Ill. 1924.

Prof. Dedrick has just given us the long promised work on flour milling which constitutes a much needed addition to the American literature of this important industry. The book includes chapters on milling principles; the various departments of the flour mill—cleaning, washing and conditioning, grinding, bolting, purification, bleaching,

analytical appliances; the application of power; mill design; and two chapters on the chemistry of wheat and flour and flour testing. The discussion of the machines used in milling, the principles of their construction, adjustment, and operation, should be especially useful to the young miller, as these discussions are developed from the fundamental principles in such a way as to enable anyone to follow through and understand the operating principles. The book is unusually well illustrated, its 400 figures covering all departments of the mill.

In the two chapters entitled "Flour Testing and Baking" (Chapter 17) and "The Chemistry of Wheat and Flour" (Chapter 18), prepared, we understand, with the collaboration of Professor Shuey, are included the outlines of the more important testing and analytical methods used in the mill laboratory. It is regrettable that this treatment of the subject did not include a somewhat more extended discussion of the interpretation of results, which is perhaps of more interest to the practical miller than the detailed description of analytical procedure, which he as a miller will probably never be called upon to carry out. These chapters should, however, constitute a convenient reference section for the mill chemist.

This book can well find a place in the library of every milling organization which makes a pretense of maintaining a library of the literature in this field, as it contains between its covers a convenient digest of much of the best thought in this field.

C. H. Bailey.

The Romance of Holes in Bread, by I. K. Russell, Editor of Baking Technology. Published by Chemical Publishing Co., Easton, Pa. 1924.

The modern chemist, who is submerged in the detailed and more or less prosaic work of the laboratory, often fails to recognize the romantic aspects of his profession. Mr. Russell has emphasized the latter aspect in a very rational and at the same time exceedingly entertaining manner in this little volume. The entire civilized world recently did honor to the memory of Louis Pasteur, but most of us are not sufficiently familiar with the extent of his brilliant contributions to modern science; contributions which are indicated in this book. The spirit of Pasteur carries on and not alone touches the fortune and happiness of each of us as individuals, but, as pointed out by Mr. Russell, provides the foundation for the scientific conduct of the great baking industry.

C. H. Bailey.

SUSTAINING MEMBERS OF AMERICAN ASSOCIATION OF CEREAL CHEMISTS

Arkansas City Milling Co., Arkansas City, Kan.
Bakeries Service Corporation, Chicago, Ill.
Banks, A. J., Ogilvie Flour Milling Company, Montreal, Canada.
Bernhard, Stern & Sons, Inc., Milwaukee, Wis.
Dunwoody Industrial Institute, Minneapolis, Minn.
El Reno Mill & Elevator Co., El Reno, Okla.
Gooch Milling & Elevator Co., Lincoln, Neb.
W. W. Hatton, c/o Messrs. Sale and Frazer, Tokio, Japan
Hecker-Jones-Jewell Milling Co., New York City, N. Y.
The Fleischmann Company, New York.
The Hoffman Mills, Enterprise, Kan.
The Ismert-Hincke Milling Company, Kansas City, Mo.
International Milling Co., Minneapolis, Minn.
Kansas Milling Co., Wichita, Kan.
Larabee Flour Mills Corporation, Kansas City, Mo.
Liberty Yeast Corporation, New York City.
Milton-Hersey Co., Ltd., Winnipeg, Canada
Minnesota State Experimental Flour Mill, Minneapolis, Minn.
Montana Experiment Station Grain Laboratory, Bozeman, Mont.
V. H. Noury & Van der Lande (Novadel Processes), Buffalo, N. Y.
Portland Flour Mills Co., Portland, Ore.
Practicum. Ltd., London, England
Purity Baking Co., St. Paul, Minn.
Societe Anonyme de Minoteries et D'Elevateurs A Grains, 24 Rue Royale, Bruxelles, Belgium
Southwestern Milling Co., Kansas City, Mo.
Wallace & Tiernan Co., Newark, N. J.
Washburn-Crosby Company, Minneapolis, Minn.
Western Canada Flour Mills Co., Winnipeg, Canada
Wichita Mill & Elevator Co., Wichita Falls, Tex.
Wichita Flour Mills Co., Wichita, Kan.

THEODORE F. ISMERT

Theodore F. Ismert, president of the Ismert-Hincke Milling Company, and honorary member of the American Association of Cereal Chmists, died at his home on the morning of September 4, 1924. One of the first flour mill operators to install a laboratory in a mill, his interest in, and appreciation of the chemist inspired him to foster the organization of this association. In recognition of this service he was made an honorary member of the association. His kindly counsel will be missed, and in his passing the association has sustained a great loss.